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AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

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The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

American Society of Medical Technologists

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The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 21 MAY-JUNE NUMBER 3

PROPAGATION OF THE VIRUS OF POLIOMYELITIS IN TISSUE CULTURE: A REVIEW

BERTTINA B. WENTWORTH, M.T. (ASCP)*

INTRODUCTION

Cultivation of the virus responsible for poliomyelitis infection has awaited first, the evolution of methods for successfully maintaining viable, actively metabolizing tissue cells outside the animal body, and secondly, the experimental selection of tissues suitable to support growth of the virus. Problems of basic tissue culture technic, nutrient fluid mediums, means of determining viral growth, and means for demonstrating the effect of virus on cells have all entered into this work. In recent years these problems have been successfully met.

Once methods for propagating this agent in tissue culture were well established, the application of tissue culture technics in the study of the epidemiology of poliomyelitis quickly led to the development of relatively simple, economical methods for the serodiagnosis of infection and for the isolation and identification of the etiological agent. The advantage of test tube cultivation over animal inoculation is obvious, especially when the principal test animal is the monkey, and tissue culture has permitted studies which would have been impossible, or at the most limited, with the methods formerly available.

HISTORY

In general there are two types of tissue culture technics, both of which have been employed in studies with poliomyelitis viruses. These may be termed the "suspended cell" and the "fixed cell" methods. In the former, finely minced fragments of tissues are introduced into flasks and float or settle into a shallow layer of nutrient medium. In fixed cell cultures, tissue fragments are planted on a substrate, which is usually a thin layer of plasma, or else are grown directly on the wall of the container.

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In 1933, Gey[1] described the use of the "Roller tube." In the Roller tube technic tissue fragments are distributed on the wall of a test tube in a thin layer of plasma, nutrient medium is added, and the tube is incubated in a slowly revolving drum. Rotation serves to expose the cells alternately to the nutrient medium and to the gaseous contents of the culture tube, as well as to insure constant mixing of the nutrient fluid during the period of growth.

It was in 1936 that Sabin and Olitsky[2] reported the first successful cultivation of the MV strain of poliomyelitis virus in suspended cell cultures of 3- to 4-month old embryonic brain and spinal cord tissues. They were, however, unable to grow the virus in non-neural tissues. The first real evidence that the virus of poliomyelitis would multiply in tissues other than those of the central nervous system was supplied by Enders, Weller, and Robbins[3,4] in 1949. They cultivated the Lansing strain in suspended cell cultures of such non-nervous tissues as human embryonic skin and muscle, embryonic intestine, and human foreskin. Upon histological examination of infected tissue fragments from their culture flasks, they noted certain degenerative changes in the cells possibly attributable to the effect of the virus.[5] In suspended cell cultures there was a decline in acid production in infected as compared with uninfected tissues, and fragments removed from inoculated cultures failed to exhibit normal cell migration when explanted to plasma cultures.

Upon microscopical examination of Roller tube cultures at low magnification, degeneration of the zone of fibroblastic outgrowth could readily be observed. Granular changes in the cellular cytoplasm were accompanied or followed by varying degrees of cytolysis, and by a diminution and eventual loss of the normal high refractile appearance of the nuclei. These degenerative changes could be prevented by type-specific immune sera and thereby provided critera for the recognition of the presence of the virus.

Various workers have since confirmed these findings as well as demonstrated growth in certain other tissues. Syverton, Scherer, and Butorac[6] obtained multiplication of virus in an even more readily available source of tissue, i.e., monkey testicular tissue. Rhodes and his associates[7] reported that human postnatal tonsillar tissue could be used satisfactorily as a source of tissue for propagation of the Lansing strain. Although not apparent in the tonsillar tissue cultures, they observed decreased utilization of glucose by infected embryonic tissues to be another manifestation of the cytopathogenic action of the poliomyelitis virus. Weller, Enders, Robbins, and Stoddard[8] obtained high titers of virus in cultures of human embryonic skin-muscle,

brain, and intestine; in preputial and testicular tissue; in kidney cortex and medulla from children; and in monkey kidney.

Even though the ability of the poliomyelitis virus to grow in tissues considered to be extra-neural was well established, it was recognized that propagation of the virus might have resulted from the inclusion of neural elements. Accordingly, Scherer and Syverton[9] obtained morphologically pure cultures of fibroblast-like cells, having no resemblance whatever to nerve cells, from monkey testicular tissue and demonstrated that pure cultures of such cells would support the growth of poliomyelitis virus. Moreover, these cells were destroyed as a result of the multiplication of virus within them, and this destructive effect could be neutralized by type-specific antisera. Following this work the same authors, in conjunction with Dr. G. O. Gey, investigated the use of the HeLa strain of human malignant epithelial cells for the cultivation of poliomyelitis virus.[10] It was found that this strain supported the growth of each of the three immunological types of poliomyelitis virus, while maintaining viability and an unaltered morphology on continuous serial passage.

The evidence that poliomyelitis virus propagates in extra-neural cells now appears unequivocal, and it can be predicted that the use of such tissues in simplified tissue culture technics will provide a means for increasingly more widespread diagnosis of poliomyelitis infection.

METHODS

I. GENERAL PROCEDURES

A. Equipment

Pyrex glassware should be employed, except for pipettes, and includes such standard laboratory items as Erlenmeyer flasks and test tubes of various sizes. Special teflon-lined screw-capped tubes* have been found advantageous for the Roller tube method, and Roux culture bottles of 1000 ml. capacity have been used for the propagation of virus in large stationary cultures.[11] When rubber stoppers are used they should be the grey variety† recommended by Parker,[12] as he found the most vigorous treatment with acids and alkalis failed to reduce the toxicity of the standard black variety. Capillary pipettes may be prepared from 8 mm. soft glass tubing, with either straight tips, or with tips bent at the proper angle for introducing tissues into Roller tubes.

B. Cleaning and Sterilizing of Glassware

All glassware should be specially cleaned either by boiling with 0.5% Ivory soap[8] or by soaking overnight in 7X detergent.‡[13]

* Supplied by the Vitis Co., 160 Ashburton St., Yonkers, N. Y.
† Supplied by the West Co., Phoenixville, Pa., of stock #124, S-41, plain.
‡ Supplied by the Linbro Chemical Co., 681 Dixwell Ave., New Haven, Conn.

After repeated rinsing in hot tap water and then distilled water, glassware may be sterilized either by autoclaving at 15 lb. pressure for 30 minutes or with dry heat at 150 degrees C. for 1 hour.

II. PREPARATION OF TISSUES[8,14]

Tissues are obtained by surgical procedures under as sterile conditions as possible. The advent of the antibiotics has reduced considerably the difficulties with bacterial contamination formerly encountered in tissue culture work, but all manipulations are best performed in a special chamber or "hood." Before use the hood is wiped out, a towel wet with Lysol is placed on the work surface and the interior is "steamed" for about 10 minutes. Ultraviolet lamps may also be used in the work area.

Mincing of tissues may be accomplished with Simms uterine scissors,[8] Number 11 Bard-Parker blades,[14] or with a Fisher press or Craigie mincer.[8] Tissues are minced to fragments approximately 1 mm. in diameter and are suspended in a small amount of nutrient medium.

After mincing, tissues may be stored under refrigeration. Temperatures of 6 to 8 degrees C. have been found more suitable than 0 degrees C. for preservation of tissue viability.[15] At 6 to 8 degrees C. oxygen is required to support the metabolism of the tissues and they should be so stored as not to restrict the availability of atmospheric oxygen.

III. NUTRIENT MEDIUM[13,14,16,19,20,21]

The nutrient medium for use in tissue cultures has usually consisted of an isotonic buffered saline solution, such as Tyrode's or Hanks balanced salt solution (BSS),[14] supplemented with various animal-derived materials, such as serum or embryonic extract. These latter components appear to provide factors, not as yet defined, which enhance growth and multiplication of cells. Simms and Sanders[17] developed an ox serum ultrafiltrate, containing a substance (A factor) which stimulated cell growth and appeared to be essential for the maintenance of living cells. This "A factor" was an acid with a molecule small enough to pass readily through a collodion membrane impermeable to proteins. The ultrafiltrate was prepared by passing serum through a collodion membrane under 600 mm. of mercury pressure. Hank-Simms medium has been very commonly employed in tissue culture work in a ratio of component parts of 3:1. Chick embryo extract, beef embryo extract, and horse serum have been variously added to this mixture and found to enhance cellular proliferation. Ledinko, Riordan, and Melnick[13] prepared chick embryo extract by cold acetone extraction according to a method described by Margoliash,[18] who felt it a better growth-promoter than fresh extract. Ledinko et al. stated that they employed

acetone-dessicated extract "so that fibroblasts with no granular lipid deposits are obtained during growth." Melnick and Riordan[19] substituted lactalbumin enzymatic hydrolysate* for acetone-dessicated chick embryo extract in their Hanks-Simms medium and obtained more luxuriant growth in Roller tube cultures with this protein-free nutrient medium than with that containing extract. Li and Schaeffer[16] have obtained very satisfactory results with bovine plasma hydrolysate† in their Medium E.

Recently Enders[20] described the growth-promoting properties of bovine amniotic fluid (BAF) and this substrate supplemented with 5% beef embryo extract and 5% normal horse serum is now routinely employed in his work when the primary objective is to achieve excellent tissue growth. His observations suggest that the amniotic fluid not only provides a physiological medium but also contains a growth-stimulating factor, or factors, although both horse serum and beef embryo extract are necessary to obtain optimum cellular proliferation without granulation. BAF also showed a more effective buffering action than did BSS-serum ultrafiltrate mixture; moreover, superior yields of poliomyelitis viruses were obtained in cultures nourished with amniotic fluid and in such cultures the minimal infecting dose of these agents was smaller.

The addition of phenol red indicator in a final concentration of 0.002% to a nutrient medium permits visual appraisal of the metabolic activity of the growing tissue, as evidenced by fall in pH. The effect of viral growth on the metabolism of tissues can then be determined by observing a decline in normal acid production. Antibiotics should be incorporated into all nutrient media in order to inhibit the growth of bacterial contaminants. Penicillin and streptomycin in final concentrations of 50 units and 50 micrograms per ml. of medium are generally employed.

Although the various media containing animal extracts and sera gave excellent results in promoting growth of tissue and of virus, it appeared that a strictly synthetic medium would be the most desirable nutrient for obtaining virus free of extraneous protein for immunologic studies. So, in 1950, Morgan, Morton, and Parker[21] introduced Medium No. 199, a synthetic medium of chemically known composition that would promote continuous growth of tissue in the absence of blood serum and embryo extract. This medium was felt to be not altogether adequate, but it supported cell life for 4 to 5 weeks. It contained 19 amino acids, 11 being dl-amino acids, and 7 of the natural l-configuration. Brown and Ackerman[22] found l-methionine to be necessary

* Supplied by Nutritional Biochemical Corp., Cleveland, Ohio.
† Supplied by Baxter Laboratories, Inc., Morton Grove, Ill., as "Travamin."

for the in vitro growth of the Lansing strain. Growth was inhibited by the addition to the culture medium of dl-ethionine, a structural analogue of the essential amino acid. When the inhibitor was withheld from subsequent fluid changes, virus growth resumed. The addition of methionine to fluids containing the dl-ethionine reduced the effect of the inhibitor. The virus appeared to be unable to synthesize methionine from betaine and homocysteine, which are its immediate precursors in other biological systems. An extension of this work[23] showed the metabolites dl-phenylalanine, adenine, guarine, and uracil also to be essential for the growth of poliomyelitis virus in tissue culture.

The Canadian workers, Franklin, Duncan, Wood, and Rhodes, have used Medium No. 199 in experiments with a variety of tissues in the cultivation of poliomyelitis virus.[7, 24] Prolonged survival of the tissues was a striking feature of the cultures in which synthetic medium was used, and this protracted survival was associated with liberation of virus into the supernatant fluid over a lengthy period. Human embryonic brain and cord survived for more than 70 days and virus was detected in the fluids of these cultures for more than 60 days. The authors felt their cultural technics could be applied to the production of large quantities of virus for use in a vaccine, for skin testing, or for study by the physiochemical methods, and that in work of this sort, synthetic Medium No. 199 has advantages over media containing animal extracts or sera, since its chemically defined composition permits fewer variations in constituents than less well defined animal materials, and cultures nourished with synthetic medium contain no antigen of animal origin.

IV. SUSPENDED CELL CULTURE TECHNIC[7, 8, 25]

Suspended cell cultures are usually prepared in 25-ml. Erlenmeyer flasks, although larger flasks have been employed for some types of investigation. Three ml. of nutrient medium is placed in each 25-ml. flask and 3 to 4 drops of tissue suspension is added by means of a capillary pipette. Syverton and Scherer[25] describe the use of a circular disc of perforated cellophane of the Beckley-OC design on the bottom of the flask, and place the minced tissue directly beneath the cellophane disc. The flasks are tightly stoppered and incubated at 35 to 37 degrees C. After an interval of 1 to 4 days to permit cellular growth, the nutrient medium is changed and 0.1 ml. virus inoculum added. The inoculum may be either infected tissue, such as a 10% suspension of monkey or mouse brain, or fluid from a previously infected tissue culture. Cultures are then reincubated and maintained by changing the nutrient fluid at intervals of 3 to 4 days.

V. ROLLER TUBE CULTURES[14, 19, 25, 26]

Various sized tubes have been used for the preparation of Roller tube cultures, including 20 by 150 mm., 16 by 150 mm., 15 by 150 mm., and 6 by 150 mm. In whatever sized tube is used, two drops of heparinized chicken plasma[27] are spread in a thin layer over the lower one-third of the tube and 6 to 10 fragments of tissue are distributed in a line along one side of the tube. A capillary pipette with the tip bent at an appropriate angle is convenient for use in introducing the tissue and arranging the fragments as uniformly as possible in the plasma layer. One to three drops of chick embryo extract[14] are added to induce clotting of the plasma and the tubes are rotated in a horizontal position to mix extract and plasma. After coagulation is complete, nutrient medium is added and the tubes are incubated at 35 to 37 degrees C. either in a Roller drum[13] or in a stationary position in an incubator. It has now been established by various investigators[11, 19, 25, 16] that equally satisfactory results may be obtained with culture tubes that are not rolled. Stationary culture tubes should be incubated on their sides in a slightly inclined position so that the nutrient fluid forms a shallow layer covering the tissue fragments.

Fluid changes are made at 2- to 4-day intervals, and 0.1 ml. virus inoculum added whenever moderate cell outgrowth is first apparent on microscopical examination. The virus inoculum is the same as that described for suspended cell cultures.

During the progress of cell growth clear areas often appear in the plasma coagulum in the vicinity of the tissue fragments. If interruption of the plasma layer is allowed to extend, the tissue fragments may become detached, leaving behind only small aggregates of fibroblasts. Such damage to the plasma coagulum can be repaired or "patched" with new plasma and chick embryo extract, but lysis can be prevented by the use of soybean-trypsin inhibitor,* as described by Fischer[28] and Morgan and Parker.[29] Concentrations up to 2.0 mg. per ml. of nutrient medium have been shown to have no appreciable inhibitory effect on tissue outgrowth. Robbins et al.[26] found that a concentration of 0.05 mg. per ml. so delayed disturbance of the coagulum that "patching" was unnecessary unless the cultures were maintained for an unusually long time.

Frisch and Jentoft[30] have described a method for obtaining a continuous supply of actively growing tissue by subculturing Roller tube cultures of monkey testicle which have been digested with trypsin. The use of trypsin to dissolve the plasma clot around tissues for transplanting purposes was first introduced by Rous and Jones[31] in 1916, and further elaborated by Vogelaar

* Supplied by General Biochemicals, Inc., Chagrin Falls, Ohio.

and Erlichman in 1934[32] and 1939.[33] After removal of the nutrient fluid, 1.0 ml. of 0.5% trypsin in Ringer's solution is added to a Roller tube culture and the tube is incubated for 1 hour at 37 degrees C., at which time dissolution of the clot is usually complete. The digested material is then washed and centrifuged and yields small clumps of cells for transplanting to new tubes. Frisch and Jentoft averaged 15 subcultures of vigorously growing fibroblasts per primary culture and propagated successful subcultures from tissues which had been kept as long as 3 months.

Robbins, Weller, and Enders[26] compared the use of Roller tube and suspended cell cultures for the cultivation of poliomyelitis virus. The chief advantages of the suspended cell cultures lay in the ease with which they may be prepared and the fact that they can furnish large volumes of infected fluid, relatively free of serum or tissue elements. Roller tube cultures are somewhat more difficult to prepare and maintain, and the medium generally used contains a considerable concentration of non-viral protein. However, in the Roller tube the degeneration of cells can be observed directly; growth of virus appears to be more rapid and the yield of virus higher, especially with small inocula. A greater number of tube than flask cultures can be prepared from the same quantity of tissue and at least one tissue, i.e., human uterus, supported the growth of virus in Roller tubes when it failed to do so in suspended cell cultures. Cellular degeneration in Roller tubes, usually apparent 1 to 3 days after virus multiplication begins, offers a more rapid and direct critera of viral growth than does the pH differential test in suspended cell cultures, which often may not be evident for 12 to 16 days after an increase in virus can be demonstrated.

APPLICATION OF TECHNICS

The cellular degeneration produced by the poliomyelitis virus in the fibroblastic outgrowth of Roller tube cultures and the fact that it is possible to prevent this cytopathogenic effect with homologous antiserum has led to the development of a quantitative neutralization test for the detection of type-specific antibody. When Roller tube cultures are inoculated with a standard dose of virus mixed with serial dilutions of serum, comparison of inoculated with uninoculated cultures provides critera for the titration of neutralizing antibody.

Strains of virus known to produce marked fibroblastic degeneration must be used in this test, since it has been shown that immunologically related strains differ in this respect. In propagation of the type 2, Yale-SK and Lansing strains in cultures of monkey testes, Ledinko, Riordan, and Melnick[34] found that while the Yale-SK strain produced a complete destruction of fibroblasts with granulation and loss of structure, Lansing strain cultures showed only slight degeneration. Younger, Ward, and Salk[35]

compared the cytopathogenic action of 4 type 1 strains, 6 type 2, and 5 type 3 strains, and found that marked differences were observed in the incubation period between the time of viral inoculation and the time of appearance of cellular degeneration; in the severity of the necrotic changes; and in the rate of spread of the virus from one piece of tissue to others in the same culture tube. In general changes produced by the type 1 strains appeared by the 6th day of incubation. Four of the 5 type 3 strains tested exhibited no cytopathogenic effect at all. The greatest variation in effect among strains was seen in the type 2 strains. Some strains produced changes by the 3rd or 4th day, while others produced no degeneration or at the most mild changes which appeared only after 12 to 15 days of incubation. It is important therefore to use a standard infectivity dose of virus, as titered in Roller tube cultures, in serum neutralization tests.

Ledinko, et al.[13] have demonstrated that a linear logarithmic relationship exists between the quantity of virus used and the quantity of serum required to neutralize the virus in a tissue culture neutralization test. The reliability of the neutralization test depends upon a number of factors, including (1) quantitative titrations of the virus and of the serum, which must be reproducible, (2) different degrees of cytopathogenic change and their unequivocal inhibition by homotypic antisera, (3) potential nonspecific degeneration of normal, uninfected tissue, and (4) the relationship between the time of reading of test end-points and the "break-through" of virus in the presence of homologous antiserum. So far as they could determine within practical limits neither the amount of tissue used nor the extent of fibroblastic proliferation prior to inoculation has any effect upon viral growth or on the results of the test.

On the basis of these findings, they recommend the following procedure for the quantitative neutralization test:

The serum to be tested is first inactivated at 56 degrees C. for 30 minutes, and then serial dilutions of serum are added to a virus suspension so diluted that the final serum-virus mixture contains 100 tissue culture doses of virus per 0.1 ml. in 10-fold serial dilutions of serum. The mixtures are incubated at room temperature for 1 hour. One-tenth ml. of each mixture is inoculated into a duplicate set of 5-day-old Roller tube cultures. Three to five tubes are left uninoculated to serve as tissue controls, and the highest concentration of serum used is inoculated without virus as a serum control. A control titration of the virus without serum is also done at the same time. All the tubes are incubated for 4 days and then examined microscopically for fibroblastic destruction. The serum and tissue controls should show no degeneration, while the virus control should show degeneration at the proper titer. The serum end-point is that

dilution of serum which completely neutralizes the virus, i.e., the highest dilution in which no degeneration is evident.

In a further study of the variables of the quantitative neutralization test, Ledinko and Melnick[36] made the following points:

(1) With 10-fold dilutions of virus, uniformly reproducible virus titers can be obtained in cultures of tissues from different monkeys. The titer in tissue culture is defined as the highest dilution of virus-containing fluid in 0.1 ml. of inoculum which produces definite tissue degeneration after a specified incubation period, that is 1+ to 4+ degeneration defined as follows:

0—No fibroblastic degeneration.

±—Some of the fibroblasts have a swollen appearance. The fibroblastic outgrowth is like that of the control tissue.

1+—First definite evidence of some granulation present. The extent of fibroblastic outgrowth is the same as that of the control tissue. The fibroblasts retain their normal structure.

2+—Granulation has become more widespread. The normal structural outline of fibroblasts is evident, but the fibroblastic outgrowth is less than that of the control tissue.

3+—Widespread granulation and shrinkage of the fibroblasts is present. Most of the fibroblasts have lost their structure.

4+—Complete destruction of the fibroblasts, with extensive shrinkage and granulation.

(2) Poliomyelitis virus is stable during the incubation period prescribed for serum-virus mixtures.

(3) Prolonged incubation of serum-virus mixtures produces a trend toward higher serum titers.

(4) Inactivation for 56 degrees C. for 30 minutes does not affect serum titers.

(5) Serum titers are not influenced by the presence or absence of complement.

(6) Virus "break-through" occurs only after 4 days of incubation and is related to the amount of antibody initially present, appearing more rapidly in the presence of smaller amounts of antibody. Renewal of antibody at each fluid replacement can prevent or further delay break-through.

(7) The order of addition of virus and serum to the culture tube is a deciding factor in neutralization. If virus is added 48 hours before serum, no neutralization occurs; when virus is added 24 hours after the addition of serum, maximum neutralization results.

These investigators obtained good correlation between tissue culture neutralization tests and the mouse neutralization test with Lansing strains of virus, and good correlation of tissue culture tests with qualitative neutralization tests in monkeys.

Although the quantitative neutralization test appears to be a reliable means for demonstrating the presence of poliomyelitis antibodies in serum, a satisfactory complement-fixation test could undoubtedly be utilized by many laboratories not equipped for doing neutralization tests. Accordingly, the use of tissue culture virus as an antigen for demonstrating complement-fixing antibodies has been investigated. Since tissue culture technics provide a means of obtaining large quantities of virus-containing fluid relatively free of extraneous protein, such antigens do not require the cumbersome and time-consuming methods necessary to purify antigens prepared from the infected nervous system tissue of animals. Svedmyr, Enders and Holloway[37,38] prepared antigens from the supernatant fluids of suspended cell and Roller tube cultures of various human tissues. These fluids were concentrated 60 to 600 times by the ultra-filtration method of Seibert.[39] When a simple medium (Hanks-Simms) was used in the cultures, the resulting concentrate was clear or slightly opalescent, colorless or brownish, and only slightly viscous at a concentration of 600 times. The use of a more complex medium resulted in a much more viscous product. It was found that heating the antigens at 56 degrees C. for 30 minutes abolished their anticomplementary action without affecting their capacity to fix complement in the presence of homologous antibodies.

Satisfactory antigen preparations were obtained by this means for each of the three immunological types of poliomyelitis virus. The greatest antigenic activity was exhibited by fluids containing the Leon strain. By "box titrations" it was determined that this antigen could be used in a concentration equivalent to only seven times that present in the crude culture fluid. Brunhilde and Lansing antigens required concentration factors of 17X and 80X respectively. These antigens were used for serum titrations in the drop method of Fulton and Dumbell,[40] whose microtechnic was employed because it is a simple, reliable test which requires only small amounts of reagents.

Paired sera from 24 cases of poliomyelitis were tested. In all of these cases virus of the Brunhilde or Leon type had been isolated from the patient's stool. The sera of 22 cases showed complement-fixing titers of 1:8 or above against homologous antigen, and in 10 cases a 4-fold or greater rise in titer was demonstrated. However, convalescent sera from all 24 patients fixed complement to some degree in the presence of at least one and most often both types of virus not found in the patient's stool (i.e., heterologous virus). In addition, five patients gave significant rises in titer to heterologous as well as homologous antigens, though when these same sera were tested for neutralizing antibodies only titers against the homologous virus were obtained.

These results suggest that the antigenic structure of the poliomyelitis virus may be complex and include group-specific as well as type-specific antigens. With human sera the complement-fixation test appears to demonstrate antibodies to group rather than type-specific antigens, even though no such results are seen when immune monkey sera is tested. Sera from this species gives type-specific results without cross reactions. However, it is not known whether the results would be the same after peroral infection of monkeys.

Tissue culture technics have not only provided means for the diagnosis of poliomyelitis infection by detection of serum antibody, but also have been applied to the direct isolation of the virus from materials obtained from patients. Robbins et al[41] obtained viral isolations from 9 fecal specimens from patients with paralytic poliomyelitis and from 5 of 6 samples from patients with clinical evidence of non-paralytic poliomyelitis. Virus was isolated from a spinal cord suspension of a case of fatal bulbar poliomyelitis, though none could be obtained from the blood. Flask cultures of infant kidney tissues were used for the isolation of the virus and Roller tubes with human embryonic skin-muscle for serologic typing of the strains.

Riordan, Ledinko, and Melnick[42] substituted Roller tube cultures of monkey testicular tissue for the flask cultures of human tissues employed by Robbins, and obtained positive isolations from 24 of 34 stool samples and from 2 human spinal cord suspensions. They, as well as Robbins, encountered difficulty with non-specific cytotoxic effects in many stool examinations. Robbins et al. prepared their fecal specimens as 10 per cent suspensions in isotonic phosphate buffer containing 500 units and 500 micrograms per ml., respectively, of penicillin and streptomycin. They compared their test cultures with a set of control cultures whose inocula had been heated to 60 degrees C. for 30 minutes in order to differentiate the cytopathogenic effect of virus from the effects of non-specific toxic substances in the fecal material. Riordan et al. used ether extraction to prepare stool specimens, but found that about 50 per cent of their samples destroyed all the fibroblasts in 24 to 72 hours. With continued incubation of these cultures the toxic effect could be eliminated by passing the fluid harvested on the eighth day to fresh cultures.

Younger, Lewis, Ward, and Salk[43] described the direct isolation and immunological typing of virus in a single step procedure by the use of inocula containing fecal suspension plus prototype antisera in Roller tube cultures of monkey testicular tissue. Their methods for preparing fecal suspensions and obtaining bacteriologic sterility were essentially those described by Howitt and Barnett[44], and their inocula consisted of 20 percent suspen-

sions by weight of fecal material in distilled water with a final concentration of 1000 units of penicillin and 10 milligrams of streptomycin. Eight-day-old Roller tube cultures were treated with 0.1 ml. of undiluted antiserum for one-half hour and then inoculated with 0.1 ml. of a 20 percent fecal suspension. (They have since found it equally satisfactory to inoculate a serum-suspension mixture as employed in the quantitative neutralization test). When toxic changes did not interfere, typical cytopathogenic changes were seen as early as the third day and were well established by the sixth day. Neutralization of the cytopathogenic effect by the homologous but not the heterologous antisera established the immunological type of the virus. In most cases identification of the virus type was unequivocal by the end of the sixth day. Rapid identification of virus was accomplished in 12 of 21 fecal specimens tested by this method. Severe toxic effects which made them unsatisfactory for testing were encountered in 8 of the remaining 9 samples. Two modifications of the procedure were tried in an attempt to solve the problem of non-specific toxicity. Assuming that toxicity and infectivity are independent variables, ten-fold dilutions of inocula were used in the hopes of eliminating the toxicity without reducing the infectivity to an ineffectual level. However, this so decreased the sensitivity of the test for viral isolation that the method was not considered practical.

So, on the assumption that larger amounts of tissue might somehow absorb, neutralize, or withstand the effects of toxic substances in the inoculum, cultures containing 0.1 ml. or 0.2 ml. of minced tissue, rather than the usual 6 pieces of tissue were employed. When these cultures were inoculated with 10 specimens known to have exhibited toxic effects in preliminary tests, clearcut positive or negative results were obtained without non-specific toxic effects. In no instance did early destruction of fibroblasts or major alterations in cellular morphology interfere with reading of the results. Thirty-seven strains of virus were isolated and typed by this modified technic, which appears to offer a satisfactory method for the rapid identification of this agent.

Much investigation in recent years has been aimed at the production of a vaccine for immunization against poliomyelitis. But many problems are involved in the production of large quantities of a safe, purified vaccine. Virus derived from infected animal tissues is both difficult to purify and difficult to obtain in large quantities. The problem of purification can be solved by obtaining virus in tissue culture fluids which are essentially free of non-viral protein and tissue derivatives. Either Hanks-Simms or synthetic Medium No. 199 are nutrient fluids which

satisfy these requirements, and both have been shown to be suitable for the production of virus in fairly large quantities[7, 11, 14].

Salk et al.[45] have prepared formalinized vaccines from Mahoney, MEF-1, and Saukett strains in Roller tube cultures of various monkey tissues nourished with Medium No. 199. The preliminary results of studies in human subjects inoculated with these vaccines have indicated that they not only induce demonstrable antibody response, but that when employed as mineral oil emulsions, they produce antibody levels which approximate, or perhaps exceed, the level induced by infection. None of the human subjects participating in this study showed any signs of illness that could be attributed to the innoculation, and there were no untoward local reactions at the site of injection. The Salk vaccine has now been employed in mass immunization of school children, and the results of this study should indicate whether a practical vaccine is at hand.

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DETERMINATION OF ENZYMES*

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A. Phosphatases

Esters of H_3PO_4 are important in the metabolism of carbohydrates, fats, and to a certain extent, of proteins. Therefore, it is to be expected that there will be mechanisms in the body which will promote the formation and hydrolysis of these compounds. These reactions, insofar as the speed with which they take place is concerned, are controlled by enzymes. The group name for such enzymes is phosphatase. It should be kept in mind that enzymes are organic catalysts produced by living cells which hasten the rate at which a given reaction will come to equilibrium. The enzyme, while at one state probably in combination with the substrate, is not used up and does not appear among the products of the reaction. Phosphatase production and distribution may be altered under pathological conditions. If a characteristic pattern of such variations can be recognized, determination of phosphatases might aid in the recognition of particular diseases.

Enzyme determinations are much more difficult than that of a single chemical group (e. g. reducing action) or of an ion (e. g. Cl^-). As far as our information goes, enzymes are proteins. Many are present in each cell and a considerable number in physiological fluids. The amount of any single enzyme is very small. An attempt to determine the amount of such an enzyme protein in the presence of many similar proteins is impossible on a routine basis at the present state of our knowledge. Therefore, recourse must be had to the determination of one of the products of the enzyme action. The amount of such product will be proportional to the enzyme present and active under the conditions chosen, and the quantity will be such as to allow reasonable ease of measurement. In addition, the analyst has a choice as to the particular component of the equilibrium mixture for which the analyses will be made. The equilibrium itself is influenced by a series of factors—pH, time, temperature, concentration of substrate, concentration of enzyme, etc. Therefore, for each method, conditions must be carefully specified and exactly followed. Results must be expressed in amount of product formed (units). If the conditions are changed even slightly, the value of the units will be altered.

Phosphatase determinations may be carried out on either serum or plasma. Serum should be used in preference to oxalated plasma if phosphate methods are used. Oxalate interferes with

* Read before the ASMT convention, June 1954, Miami Beach, Florida.

the development of the phosphate color (Standard Methods of Clinical Chemistry. Academic Press, Inc. N. Y. 1953, p. 83).

The stability of phosphatase in serum varies with the type. Alkaline phosphatase activity increases significantly on standing. This increase may amount to 10% after 24 hours refrigeration and to as much as 20% after 4 to 6 hours at 37° C. (Cantarow & Trumper: Clinical Biochemistry. Saunders. Philadelphia. 4th Edition, 1949, p. 195). Therefore, determinations should be done as soon as possible and samples of serum should never be preserved more than 18 hours. (Woodard: Cancer Research **2,** 497-508 [1942]).

Acid phosphatase is much less stable, particularly if the pH is elevated and the temperature is not lowered. There is significant loss of activity in 30 minutes at 28° C. from samples of sera giving high acid phosphatase values. (Woodard: J. Urology, **65,** 688-90 [1951]). Presumably, if a serum sample is frozen immediately following the bleeding of the patient, its acid phosphatase activity will remain constant for some hours. In view of the above statements, it seems evident that if accurate serum phosphatase levels are to be obtained, the patient must either come to the laboratory to have the blood drawn or freezing facilities must be available where the patient is. In either case the analysis should be begun promptly and the blood may be transported only minimal distances.

There are at least 3 types of phosphatases which must be taken into account: (1) Alkaline phosphatase with optimum activity at about pH 9.3. Occurs in blood serum, kidney, intestinal mucosa, and other organs, but is associated primarily with osteoblastic activity in connection with bone; (2) acid phosphatase with optimum activity at about pH 5. Occurs in blood serum, prostatic secretion, kidney and other organs; (3) acid phosphatase with optimum activity at pH 6. Found in mammalian erythrocytes.

The laboratory problem is to differentiate between the three and to provide the most accurate possible measure of activity which is consistent with providing prompt reports to the clinician. Alkaline phosphatase is no particular problem, since acid phosphatase is completely inactivated by incubation at pH 8.0 or above for one hour (Woodward: J. Urology **65,** 688-90 [1951]). Clinical interest is in the phosphatase which has its optimum activity at pH 5.0 and its separation from the similar enzyme which occurs in the red cell is rather difficult. Given blood samples in which no hemolysis has taken place, the problem is theoretically non-existent. This, however, is difficult to ensure and in addition, the laboratory would like to be able to salvage hemolyzed samples. As a result, attempts have been made to find specific reagents which will inhibit either the prostatic or the

red cell enzyme. Examples of the former are 40% ethyl alcohol, and of the latter 0.5% formalin (final concentration in each case) (Delory, Sweetser, and White: J. Urology **66,** 724-33 [1951]. Bensley, Wood and Lang: Am. J. Clin. Path. **18,** 742-44 [1948]). A solution containing approximately 0.02 M L-tartrate is said to inhibit the activity of the prostatic enzyme (Fishman and Lerner: J. Biol. Chem. **200,** 89-97 [1953]).

The results of serum phosphatase determinations are of use in the recognition of certain pathological conditions. Serum alkaline phosphatase activity is above normal in active rickets, hyperparathyroidism, osteitis deformans (Paget's Disease) and is occasionally elevated in generalized osteoporosis, marked hyperthyroidism, healing fractures, osteogenic sarcoma and metastatic carcinoma involving bone. Claims have been made that alimentary hyperglycemia and high carbohydrate diets in general result in increased activity. There is some evidence that malnutrition, anemia, senility and high protein diets cause a decrease of alkaline phosphatase activity in the serum.

The determination of serum acid phosphatase has been primarily of value in the detection of carcinoma of the prostate with metastases. Abnormally high serum acid phosphatase activity, up to several hundred times normal, is usually but not invariably associated with metastatic carcinoma: About 85% of such cases show high values while only some 5% of false positives are obtained (Herbert: Quart J. Med. **15** (new series), 221-41 [1946]. Arner and Swedin: Acta chir. Scandinav. **97,** 135-47 [1948]). Prostatic secretion is rich in acid phosphatase and the escape into the blood stream of a fraction of a milliliter would be sufficient to explain the elevated values found in samples of serum. It has been suggested that since other channels are not available, secretion formed in the metastatic centers of cells of prostatic origin escapes directly into the blood stream. If this should be the explanation, one can understand why malignant growths in the prostate gland itself rarely cause high serum acid phosphatase levels.

In the choice of a method to be used for the determination of phosphatase activity, one is confronted with a variety of substrates. Each has advantages and disadvantages. The first of such materials to be employed was Na B-glycerophosphate, an ester closely related to naturally occurring substances. In this case, the amount of inorganic phosphate liberated by the action of the enzyme, is measured. Reagents for and experience in the determination of inorganic phosphate is available in practically every clinical laboratory. On the other hand, a certain amount of inorganic phosphate is always present in biological fluids including blood serum. If allowance is not made for this preformed phosphate, it may lead to confusion.

When glycerophosphate was unavailable during World War II, many laboratories turned to a completely synthetic substrate, phenyl phosphate. In this case the phenol liberated by phosphatase may be measured. The picture here is not complicated by the presence in the blood of phenol except in traces. The phenol solution of known concentration for use as a standard is, however, not easy to prepare, the substrate solution is not too stable and the preparation of the color reagent for the phenol determination is rather time consuming.

Two substrates have been suggested, phenolphthalein phosphate and p-nitrophenyl phosphate which produce colored materials when hydrolyzed. This simplifies the determination of enzyme activity.

The author's personal preference is for a method which involves determination of phosphate (Shinowara, Jones and Reinhart: J. Biol. Chem. **142**, 921 [1942]. Hawk, Oser & Summerson: Practical Physiological Chemistry. Blakiston, Philadelphia. 12th Edition. 1947, p. 584). The latter reference employs the Fiske & SubbaRow technique for the determination of phosphate. This again is personally preferred by the author.

A Unit = amount of P liberated by 100 ml. of serum in one hour at 37° C. The incubation time may differ for other units.

Alkaline phosphatase normal values (Bodansky Units from determination of P)

1.5 to 4.0 units average 2.7 for adults
5.0 to 12 units average 8.0 for children

Acid phosphatase normal values (Bodansky units)

0.0 to 1.1 units

King-Armstrong Units (from determination of phenol) are approximately 1.8 times those given above, although no fixed relationship pertains between the two types of units.

B. Pancreatic Enzymes

Determinations may be carried out on serum (amylase and lipase) or on material collected by means of a duodenal tube (amylase, lipase, and proteinase).

If serum is used, the analyses should be carried out promptly. As was pointed out above, enzyme solutions are quite unstable. If duodenal material is to be analyzed, it must be carefully collected (Bauman: The diagnosis of pancreatic disease. Lippincott, Philadelphia. 1949). The pancreas must be stimulated with secretion of mecholyl. The admixture of gastric secretion must be prevented and the container for the duodenal sample must be cooled with ice.

Amylase activity is determined by action on a suspension of especially prepared starch (Hepler: Manual of clinical laboratory methods. Thomas, Springfield, Ill., 1949, p. 316). The reducing carbohydrate resulting from the action of the enzyme may be

determined by the sugar method in use in the laboratory. One unit of amylase activity = mg. sugar (calculated as glucose) produced by 100 ml. of serum or duodenal contents in 30 minutes at 40° C. Normal serum values are 80 to 150 units. High figures are obtained in the serum during the early stage of acute pancreatitis. It should not be inferred from this discussion that normal serum amylase activity arises from the pancreas. It is present following surgical removal of this organ.

Lipase can be determined using tributyrin as the substrate (Comfort: Am. J. Digest. Dis. and Nutrition **3,** 817-21 [1937]). Units in this case are milliliters of 0.05 N NaOH necessary to titrate to the phenolphthalein endpoint, the fatty acid liberated by 1 ml. of serum or duodenal contents.

If duodenal contents are incubated with a solution of casein, digestion of the protein will occur, the extent depending on the proteolytic activity of the sample. The amount of digestion can be determined by precipitation of the undigested protein with CCl_3COOH and an assay of the filtrate for total nitrogen. The usual non-protein nitrogen technique serves for this purpose. The result is reported in mg. of NPN formed by 0.02 ml. of duodenal contents (Free and Myers: J. Lab. Clin. Med. **28,** 1387-98 [1942-43]).

This discussion has served to point out certain precautions which must be observed if reasonably accurate determinations of enzyme activity are to be obtained on samples of biological material. Methods in current use for such determinations have been indicated.

PROPOSAL FOR THE DISTRIBUTION OF A CERTIFIED STANDARD FOR USE IN HEMOGLOBINOMETRY*

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There is probably no procedure more commonly used in clinical laboratories than hemoglobinometry and few that are less satisfactory in their results. The deficiencies may reside, in part, in inadequacies of the chosen method. In larger part, however, they result from manipulative errors in the measurement and processing of the samples of blood. The latter involve questions of technical proficiency which are entirely within the control of the analyst himself. When, however, he converts his observations—gasometric or photometric readings, measurements of specific gravity, etc.—to grams of hemoglobin in 100 ml. of blood, he must depend upon his own calibration or a calibration supplied by an instrument maker, both procedures involving a conversion factor taken from the literature. To be satisfied that the calibration remains valid for the instrument, reagents, and technical routines in use in his laboratory, he should periodically undertake a precise and time-consuming series of standardizations. If a uniform hemoglobin standard were available nationally and a single method of analysis widely practiced, the individual laboratory would then have assurance not only that its results would be comparable from month to month, but that they would also be comparable with those from other laboratories employing the same standard. Inconsistencies in results that persisted under these conditions could then be clearly attributed to technical and manipulative errors.

The purpose of this report is to describe a plan for the distribution of a certified hemoglobin standard and to advocate the wide adoption of a recommended analytical procedure. The use of both the new procedure and the standard is recommended. At the same time it is pointed out that the standard may be employed for the calibration of other methods of hemoglobin analysis in laboratories that do not choose to adopt routinely the advocated photometric procedure.

In 1941 the British Medical Research Council instituted an extensive study of the problem of standardizing hemoglobinometry and emerged with (a) a recommended analytical procedure, (b) a simple hemoglobinometer for general use, and (c) a certified national hemoglobin standard for distribution to cooperating laboratories.[1,2,3,4]

* Presented in part at the meeting of the American Association of Blood Banks, Washington, D. C., September, 1954.

In the United States the initiative was taken by the Army Medical Service Graduate School with a limited field trial[5] of a cyanmethemoglobin solution proposed by Dr. David L. Drabkin. The success of the Army plan so impressed the Hematology Study Section of the National Institutes of Health that it requested the National Research Council to explore the possibility of establishing a National Hemoglobin Standard for general use throughout the country.

To this end the Academy-Research Council established an **ad hoc** Panel under the Subcommittee on Blood and Related Problems of the Division of Medical Sciences. This Panel has sought the cooperation of the College of American Pathologists, the American Society of Clinical Pathologists, the American Association of Blood Banks, the Department of Defense, the Veterans Administration, the National Institutes of Health, and the National Bureau of Standards. It has also maintained close liaison with the Committee on Hemoglobinometry of the Medical Research Council of the United Kingdom and with the National Research Council of Canada.

The Panel gave serious consideration to the British plan, but decided that, in respect to simplicity and adaptability, the cyanmethemoglobin method[6,7,8,9,10,11] adopted by the U. S. Army would be more suitable for use in the United States and Canada. It was agreed that the choice of a solution of some form of hemoglobin as a standard for hemoglobinometry was both logical and direct. In contrast to a glass standard, it would have the advantage of adaptability to a variety of photometric instruments and cuvettes. Among the forms of hemoglobin well adapted to photometry, cyanmethemoglobin has outstanding advantages. It has been shown that solutions of this pigment are stable for years when preserved at refrigerator temperatures.[12] The absorption band of cyanmethemoglobin in the region of 540 mμ is broad rather than sharp, so that its solutions are suitable for use in filter type photometers as well as in narrow band spectrophotometers.[13,7] Finally, all forms of hemoglobin likely to be found in blood, with the exception of sulfhemoglobin, are quantitatively converted to cyanmethemoglobin upon the addition of a single reagent.[7]

Recommendations

On the basis of these considerations, the Panel reached agreement on the following recommendations:

1. There shall be a Standard of Reference in the form of a preparation of crystalline human hemoglobin prepared by the method of Drabkin.[14,15,16] The acceptable criteria for this preparation shall be that a solution containing 1 milliatom of hemoglobin

iron per liter shall have a milliomolar extinction coefficient of 11.5 at a wave length of 540 mμ, when measured as cyanmethemoglobin.[13, 7] Certification of this Standard of Reference shall rest upon the results of spectrophotometric measurements and of analyses for iron made independently by the National Institutes of Health, the Army Medical Service Graduate School, the National Bureau of Standards, and Dr. Drabkin's laboratory at the University of Pennsylvania.

Professor King of the Postgraduate Medical School in London will also characterize the Standard of Reference both chemically and spectrophotometrically and compare the results of its use with determinations of hemoglobin employing the British Standard.

2. The iron content of hemoglobin shall be accepted to be 0.335 per cent.[17] This value for iron is the traditional figure used in this country and is in substantial agreement with that adopted by the British. It corresponds with an equivalent weight for hemoglobin of 16,700 per atom of iron and with an oxygen capacity of 1.34 ml. per gram of hemoglobin.[17]

The adoption of an agreed figure for the iron content is necessary in order that the spectrophotometric measurements in terms of iron may be translated into grams of hemoglobin. Should any change be made in the future in the accepted values for the extinction coefficient of cyanmethemoglobin and the iron content of hemoglobin, results based on the use of the above standard may be readily recalculated.

3. There shall be a Standard for Distribution in the form of a certified solution of cyanmethemoglobin which shall be prepared directly from the Standard of Reference. The Standard for Distribution shall be packaged as three separate solutions containing certified concentrations of approximately 20, 40, and 60 milligrams of hemoglobin in the form of cyanmethemoglobin per 100 ml.

These three solutions will correspond to 1 to 250 dilutions of blood containing approximately 5, 10, and 15 grams, respectively, of hemoglobin per 100 ml. After bottling, samples of these standard solutions will be spot-checked for correctness of optical density by the four analytical laboratories that have been designated above. The batch will then be certified and distributed to cooperating laboratories by designated national agencies.

4. In conjunction with the use of the proposed standards, it is recommended that clinical laboratories consider the adoption of the cyanmethemoglobin method of hemoglobin determination described by Drabkin.

The use of the cyanmethemoglobin method follows from the concept that it would be logical to adopt a method of

analysis which converts hemoglobin into the same pigment as that used in the standard. It does not, however, preclude the use of the cyanmethemoglobin standard for the calibration of other methods of hemoglobin analysis which may be in routine use in some laboratories. However, it should be realized that such a procedure may lead to some loss of accuracy.

The cyanmethemoglobin method employs a single solution containing potassium ferricyanide and potassium cyanide, which converts the hemoglobin in blood quantitatively to cyanmethemoglobin. The ferricyanide converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with potassium cyanide to produce the stable pigment cyanmethemoglobin. These two reactions are rapid and stoichiometric.

There should be no reluctance to employ this standard and reagent because they contain cyanide. The concentration of cyanide in the reagent that is proposed for use is only 52 milligrams of potassium cyanide per liter. Its lethal dose for man approaches four liters.[18] Most clinical laboratories use for the determination of uric acid, a reagent containing 50 grams of this salt per liter. In view of this and of the fact that laboratories of clinical pathology are disciplined in the use of such dangerous materials as isotopes and virulent pathogens, it would seem that the handling of the proposed reagent constitutes a quite negligible hazard.

The Panel also agreed to undertake a field trial of one year's duration using the Standard for Distribution described above. It is now felt that the plans for this field trial have progressed to the point where the participation of laboratories desiring to cooperate may be invited. The standards will be prepared by Dr. David L. Drabkin, and distributed without charge to clinical laboratories on application, provided they will agree to meet certain minimum requirements for participation, as follows:

1. To conduct and report at three-month intervals, for one year, measurements of the actual photometric readings of the three standard solutions in the photometer routinely in use for hemoglobin measurements in that laboratory.
2. To cooperate in answering a simple questionnaire designed to furnish information on the influence of various factors on the results of the hemoglobin determinations which will assist the Panel in its long range plans for making this standard available on a national scale.
3. To cooperate in the analysis and reporting of (a) an unknown solution of cyanmethemoglobin, and (b) an unknown sample of blood.

The Standard for Distribution, consisting of the three solutions described above will be packaged as a single unit. Details

of the procedure for the determination of hemoglobin as cyanmethemoglobin, as well as details of the procedure for calibrating anothr method in terms of the cyanmethemoglobin standard, will be furnished with the standard.

Distribution will be made to civilian laboratories by the College of American Pathologists, 203 North Wabash Avenue, Chicago, Illinois; to military and government laboratories by the Army Medical Service Graduate School, the Navy Bureau of Medicine and Surgery, the Air Force Surgeon General's Office, and the Veterans Administration†; and to laboratories in Canada through the Division on Medical Research, National Research Council, Ottawa, Ontario, Canada. Cooperating laboratories are requested to apply to the distributing agency with which they are most closely associated. Because of limitation in the number of sets of the standard available, distribution will be determined by priority of application and willingness to comply with the conditions listed above. Application for standards will assume acceptance of these conditions.

It is estimated that the Standard will be ready for distribution by April 15, 1955.

This plan has been drafted by the **ad hoc** Panel on the Establishment of a Hemoglobin Standard of the Division of Medical Sciences, National Academy of Sciences—National Research Council.

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Hints: From B. G. Mail, Doctors' Clinic, Salem, Oregon

For ten years the art of making good blood agar plates has rewarded me with far more disappointing failures than successes. I either added the whole blood to the too hot melted blood agar base and acquired completely hemolized blood plates, or I waited too long before I added the blood and the resultant plates resembled lumpy mashed potatoes. To the majority of the technologists "spare time" is not included in the working day's activities. Thus the above results are frustrating.

One day it was necessary for me to interrupt my blood agar plate task just as the agar had been completely melted. Without giving it much thought, I thrust the flask of melted agar into the 56 degree water bath and forgot it for nearly an hour. At the end of that time I removed it, added the whole blood and poured my plates, expecting the usual failure. Much to my surprise they were perfect. No hemolysis and no lumps!

I have tried the same trick a few times since, leaving the melted agar in the water bath only long enough to assume the 56 degree temperature, and my previous failures have changed to 100 per cent successes.

Being employed in a clinic where sterile whole blood isn't always available at the instant blood plates are needed, I've tried another trick with no adverse results. I've taken 8-10 discarded tubes of oxalated blood and poured them into the melted blood agar base. The oxalate tubes were not sterile before receiving the patient's blood—nor were the syringes used in collecting the blood sterile. I used the above method because I had no other choice at the moment and the plates remained free from contaminants for several days.

THE CHANGING CLINICAL PICTURE OF SYPHILIS

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Syphilis has been described as a disease peculiar to man, essentially chronic in its course, intermittent in character, and either acquired or congenital. It is capable of affecting any tissue of the body. It was characterized as being ubiquitous, insidious and disastrous, a picture which is now in certain countries being rapidly changed by new methods of both treatment and control. The clinical picture of syphilis has been greatly changed in the past decade. While this is currently attributed to improvements in the modern method of treatment, and while this plays the larger role in the immediate post-war trend, we must not lose sight of the fact that treatment and diagnostic methods developed in the early part of the twentieth century have contributed greatly toward this end. In order to better understand the current attitude and the situation which exists today, let us turn now to the record of the past.

Syphilis has been one of the greatest and most persistent plagues that ever attacked mankind. The history of syphilis is as interesting as the story of any disease which ever afflicted the human race. The name by which it is now designated is that of the hero of the poem "Syphilis sive Morbus Gallicus," by the Veronese philosopher, Girolamo Fracastoro. We find our first reference to the disease in the early part of the sixteenth century in Spain, following the return of the Columbian expedition to the New World. The rapidity with which it spread throughout Europe in the sixteenth century, the very large segment of the population which was affected, and the virulence of the disease during that period, is such that we must assume that mankind held less natural immunity for the disorder than is the case today. The disorder spread first to France and Italy. Soldiers of Ferdinand were sent to Naples to garrison a town which was the object of attack of the armies of Charles VIII of France. It is said that Spanish mercenaries accompanied the French invaders. In any event, the epidemic which encompassed the city was referred to by the natives as the "French pox," and by the French soldiers as the "Neapolitan Disease." It has, of course, always been fashionable to blame one's troubles on someone else.

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* Revised from an illustrated lecture on the clinical picture of syphilis presented before the American Society of Medical Technologists, June 17, 1954.

Many contended that syphilis was not introduced from the New World, but we will not enter that controversy, but continue our story of the rapid spread to most of the countries of Europe, from whence it found its way to India, China and finally Japan, probably carried by the Portuguese navigators whose voyagers played so prominent a role in this century of exploration. The disease was decidedly more malignant than that which we know today. In this century we seldom have the opportunity of seeing a case of precocious malignant syphilis which resembles the description of the disease which has been handed down to us from the past. It is interesting to note that mercury was early introduced into the treatment of syphilis, and that it continued to be one of the major therapeutic weapons for more than four hundred years. Unfortunately, through lack of adequate knowledge of its undesirable side effects, it fell into disuse, and was avoided by many physicians, though its value was eventually recognized when used with appropriate controls.

John Hunter, the great eighteenth century English anatomist and surgeon, still confused the two common venereal diseases, syphilis and gonorrhea, and it remained for Ricord and Bell, by careful clinical studies and observations, to establish the disorders as separate clinical entities. The name of syphilis was not widely adopted as a designation for the disease until the latter part of the eighteenth century. The nineteenth century found the syphilolographer in a preeminent position, and only the clinician who had had years of experience in examining thousands of patients with primary and secondary and late lesions of syphilis, could qualify to make the diagnosis, for it was a diagnosis which could be made only on clinical observation, and for which there was no laboratory support. The cause of syphilis eluded the investigations of its students, and was not resolved in the early bacteriological era in the latter part of the nineteenth century. It remained for Schaudinn and Hoffman to demonstrate the organism in 1905, and for Wassermann to adopt the complement fixation test to the diagnosis of the disease in 1907, and for Ehrlich to announce his "therapia sterilisans magna" in 1909. To this we may add the introduction of bismuth by Sazerac and Levaditi, which ultimately displaced mercury, and the development of penicillin in the closing years of the last war. It has largely displaced the heavy metal therapy for this disease in all countries where it is available and inexpensive.

Medical technologists who have had the opportunity of working in a venereal disease clinic may be familiar with some of the clinical aspects of syphilis. This is particularly true of those who have had experience in darkfield examination of the infectious lesions of the disease. The majority, however, are seldom

called upon in this capacity, and their point of contact with the disease is frequently limited to the performance of a serologic test for syphilis, of one or another type, designed to detect reagin in either the blood or the spinal fluid.

A patient with syphilis does not always have a positive blood test and, conversely, a patient with a positive blood test does not always have syphilis. The exceptions which make this statement true can best be explained by first reviewing the clinical course of the disease, and then by pointing out certain deficiencies in the widely used serum test for syphilis. From the time of innoculation of the subject, whether it be a laboratory animal, or man, there exists a period before the development of the initial clinical manifestation of the disease, which may be termed the primary period of incubation. In the interest of brevity, we will confine our story to clinical syphilis in man, and state that this period varies and may be as short as a week, or under rare and exceptional conditions, as long as two or three months. The mean average time, however, is twenty-six days and, at the end of this period, the initial lesion manifests itself as an erythematous papule, which may rapidly increase in size and become indurated and eroded on the surface to form the primary lesion of syphilis, which is called the chancre. During all of this period, all of the serum tests for syphilis are negative, and yet the individual has been innoculated with the disease, his blood and body tissues contain the spirochetes, and he can transmit the disease if during this period he is used as a blood donor. There is no method of detecting the presence of the disease in the primary incubation period. Since, however, this individual has no clinical lesions on the skin or mucous membranes, he is not a source for contagion by ordinary contact.

The incubation period is terminated by the appearance of the initial lesion which at first is small. This lesion may remain small and go undetected. The large number of cases of latent syphilis, in which no previous diagnosis of the disease has been made, indicates that this may be as common as it is for the individual to run the classical course for the disease. Due to anatomical differences, a concealed or occult chancre is the more common picture in the female. More than 90% of all of the visible primary lesions ocur on the genitalia, and less than 10% of the lesions are extra-genital. Of these, only a small fraction, estimated at less than 1%, are accidentally acquired.

The typical Hunterian sore is an indurated papule, having the approximate size of a split pea, and the consistency of a flexible piece of cardboard, which is fixed to and eroded through the overlying skin. It occurs on the prepuce, or in the coronary sulcus of the penis, and it increases rapidly in size after its

appearance, and then slowly regresses, but is still present at the time the secondary eruption appears. There are many variations in the size and character of the initial lesion of syphilis. These depend upon its location, the presence, or absence, of secondary infection of various types, the resistance of the host to the invading organism, and perhaps even the strain of spirochete and the number of organisms which are introduced at the site of innoculation. For the most part, extra-genital lesions tend to be larger and more inflammatory in character, atypical in appearance, and therefore a more likely source for diagnostic error.

The complement fixation tests for syphilis which were first described, lacked the sensitivity of the tests which are in use today, and it was not until the fourteenth, and sometimes the twenty-first, day after the appearance of the initial lesion, that the titer of reagin in the blood had developed to a point where the test was reported as positive. During this period, from the appearance of the initial lesion until the serological test for syphilis was positive, the diagnosis could be made, or confirmed, by the darkfield examination. This examination demanded a certain amount of skill and experience on the part of the technician, or clinician, in identifying Treponema pallida, and differentiating it from other spirochetes which might be associated with it. These early lesions were designated as sero-negative primary lesions of syphilis. With the introduction of the flocculation tests, and the increase in sensitivity in both flocculation and complement fixation tests, the interval between the appearance of the primary lesion, and the development of sufficient reagin to give a positive serologic test for syphilis, was progressively reduced. Now, with some of the more sensitive tests, the disease can be detected almost as soon as the lesion has developed sufficiently to suggest the clinical diagnosis of chancre. These extremely sensitive tests, however, must be accepted only as a confirmatory tool for special problems, and not used as a routine procedure in the diagnosis of syphilis. They may be helpful if the lesion is an atypical one, or if local treatment has been applied, or the patient has received an antibiotic. In such cases a darkfield examination may no longer be successful.

The secondary incubation period extends from the time of appearance of the initial lesion until the onset of the generalized eruption. This may be as short as two or three weeks, or as long as three or four months, but it averages 44 days. At the end of this period, by which time the primary lesion may have decreased in size and started to heal, there occurs a generalized eruption which at first is characterized by faint, erythematous, macular, maculo-papular, lesions. This may be so faint as not

to be evident, except to the trained observer. The secondary eruption of syphilis is so protean in its manifestations that it may mimic any of the contagious exanthems, or many of the drug eruptions. It may fade within a matter of days, or within a few weeks, without producing any further evidence of the disease, or it may be followed by the appearance of an extremely florid, severe eruption which will last for many weeks. The more pronounced secondary eruptions have more diagnostic characteristics, but even so, they may closely resemble many other disorders. The trained clinician will observe certain features which will arouse his index of suspicion, and examination may reveal a generalized lymphadenopathy, enlargement of the spleen, a concomitant sore throat, the presence of eroded patches in the mouth, congestion and enlargement of the tonsils, falling hair, tender spots over the shins or skull, and many other less common findings which formerly constituted the hall-marks of syphilis on which the clinical diagnosis was established.

During all of this period, from the development of the primary lesion to the appareance of the secondary eruption, the serum test for syphilis should be strongly positive. It becomes more strongly positive when the secondary eruption is fully developed, and the test remains persistently positive after the secondary eruption has involuted. The clinical syphilographer had two well established dictums concerning secondary eruptions. First, that it is never vesicular, and, second, that it is never pruritic. To each of these there is an exception to prove the rule. Congenital syphilis in the infant may produce a bullous eruption on the palms and soles, and follicular syphilides, which are more common in the colored races, may cause itching.

Secondary eruptions which begin with a faint macular or papular generalized eruption may change in character if untreated, or they may disappear, leaving no manifestations of the disease. Involution may be followed by a series of relapsing secondary lesions on the skin and mucous membranes, and these may be associated with, or accompanied by, visceral or neuro-recurrences, which are most frequent in the early years of the syphilitic infection. For the most part, infectious relapse occurs during the first two years of the disease. Finally, the lesions of secondary syphilis become less numerous, restricted to certain areas, and they begin to take on the characteristics of late lesions of the disease. In rare instances the eruption assumes fulminating characteristics, with extensive destructive ulceration, to produce the picture of malignant syphilis. This form of syphilis is associated with the lack of development of specific resistance to the disease on part of the host, and is often

not accompanied by positive serological tests for syphilis. Fortunately, such cases are now exceedingly rare.

With the close of the secondary period, and the disappearance of visible manifestations of the disorder, the course of the disease is no longer predictable, but is diversified, probably in accordance with the resistance of the host, or less likely, in response to the strain of the organism involved in producing the infection, or with some secondary infection, or illness, which supervenes to alter the resistance of the host. In any event, he enters upon a period of latency in which there is no clinical manifestation of the disease.

It is in this phase of latency, when there is no clinical evidence of the disorder, that the serological tests for syphilis are most valuable, for it is possible to detect these latent infections in the patient before he has been seriously harmed by the presence of the invading organisms. Not all of those who have been infected will be damaged by the disease, even though it persists and remains untreated. Some of them may live an entire life span with a positive serum test for syphilis and never develop clinical evidence of the disease. In fact, a considerable number have been shown to undergo sponstaneous cure, and show neither clinical evidence of the disease, nor serological evidence, even when carefully examined a good many years after their initial infection. This interesting clinical and statistical study published by Bruusgaard, from Norway, has given us much valuable information concerning the course of untreated syphilis during the present century. This can be attributed to either natural attenuation in the virulence of the organism, or a natural increase in resistance of the race to the infection. This valuable and significant study disclosed that half of those who lived for twenty years after the infection, which was untreated, did so without development of active lesions of any kind, and in 14% the sole remaining evidence of the disease was a positive serum test for syphilis. It must be pointed out, however, that central nervous system relapse was two to four times more common than in those who received treatment, and that cutaneous relapses were approximately twenty-five times more frequent in the untreated group. In other words, with adequate treatment, approximately 77% remained free of difficulty, and with no treatment, only 24% to 36% remained free of symptoms.

The largest group of individuals with syphilis will enter a latent phase in which the serum test for syphilis may remain positive indefinitely, or throughout life. Once the disease is well established, and a period of several years has elapsed, only a few will become serologically negative following treatment for syphilis, either by the older methods, or by the use of penicillin

and modern therapy. This stage of latency may persist throughout life, or may be broken at any time with the development of tertiary, or third stage, manifestations of syphilis. This may follow within one or two years of the early manifestations of the disease, or not manifest itself until many years after the original infection was established. It may be confined to a banal, localized, minor, gummatous infiltration of the skin which will be recognized by the experienced syphilographer, or by the clinician with a high index of suspicion. The serum test for syphilis in such cases is almost always positive, though the titer may not be as high as that found during the florid secondary eruption. The host may develop visceral manifestations of the disease of such character that they do not produce clinical symptoms, and yet there will remain pathological evidence of the disease in the aorta, liver, or central nervous system, which is not of sufficient extent to reach the clinical horizon. On the other hand, these small, latent lesions may gradually increase in severity until they produce extensive, ulcerating, destructive eruptions on the cutaneous surface, which are so classical as to constitute a walking sign-board of the disease. They may produce destructive lesions which involve the mebranous bones of the nose, face and the skull, they may invade the stomach, or liver, diffusely, or involve any of the viscera to produce a clinical picture which may be either characteristic, or conversely, extremely confusing. The tertiary picture may be quite benign, but on the other hand, it can destroy the individual, depending upon the location and character of the involvement. General paresis, with insanty, locomotor ataxia, with involvement of the spinal cord, and certain of the cardiovascular pictures, are late manifestations of the disease.

The advent and extensive use of adequate treatment for syphilis has markedly altered the clinical picture of this disease. We are reaping today the benefits of adequate therapy over an extended period, from standard treatment which was administered ten, twenty and thirty years ago. The late complications of syphilis have been reduced in number and frequency by the modern attacks upon the disease, even before the introduction of penicillin. We anticipate that this new method of treatment, which is far safer, and much less expensive, will result in further diminishing the late ravages of the disease, but only an extended period of observation can confirm this desirable end. While it appears that the incidence of early infection is being markedly diminished in those countries where adequate medical care and treatment is available, we must not lose sight of the fact that it is still one of the most widespread diseases in the world, that it still infects millions of people and causes widespread damage, when adequate control is not maintained. There are many coun-

tries throughout the world, in Asia, Africa, Polynesia, and the Carribean area, where large segments of the population receive little or no modern medical care, and the incidence of treponemal disease, including syphilis, remains at a high level.

Since in many cases it is impossible to determine the dividing line between late secondary lesions which show tertiary manifestations, and relapsing lesions which simulate secondary eruption, it has become the modern practice to designate syphilitic manifestations as either early, when the duration is less than three years, or late, when the duration is greater than three years. The older division of syphilis into three stages, which was devised by Fournier, is no longer in general use, and yet we refer to it again and again in discussing the clinical manifestations of the disease, the serological results which are obtained, and the alterations in the tissues which occur as a result of the reaction of the host to the invading parasite.

Having developed the clinical picture of syphilis in skeleton form, let us return now and correlate the serological picture with the clinical manifestations of the disorder, and with the changes which take place in the tissues of the host. When the first Treponema pallidum enters the host, it does so without producing any evidence at the site of entry, where it grows in increasing number and invades the surrounding lymphatic tissue, and eventually reaches the regional lymph nodes and becomes a generalized invader throughout the body before it is manifest at the site of innoculation. All of this happens before any of the serological tests become positive. In this stage the tissues of the host do not react to the invading organisms, but after they have been present for variable periods of some three to four weeks, or longer, an inflammatory, infiltrated type of lesion develops at the site of innoculation, at or about the time sensitive serological tests for syphilis are reported as positive. The amount of reagin in the blood continues to increase with the increasing number of organisms throughout the body, and the increasing resistance of the host to the invading parasites, which reaches a peak with the exploding secondary eruption. There have been changes too in the character of the cellular resistance to the organism, and the histologic picture, which was initially an inflammatory one, has taken on tuberculoid characteristics, first at the site of the involuting chancre, and later with the passage of the initial secondary eruption, the relapsing secondary lesions become less inflammatory, and finally tuberculoid in character, to resemble the picture of the late gummatous lesions. In some cases the picture develops into a sarcoid-like reaction, presumably with increasing resistance of the host to his disease. We are dealing now with a host whose tissues have entered a state of altered re-

activity, and acquired resistance to a disease which did not previously exist. This altered state of reactivity is an allergic type mechanism which is seen in many chronic infectious diseases, and is especially well delineated in the course of syphilis.

For almost fifty years we have supplemented our clinical ability in making the diagnosis of syphilis by various laboratory tests which measure the amount of reagin in the blood. These tests have been progressively improved by increasing their sensitivity, and by attempting to arrive at an optimum point between sensitivity and specificity. We have become aware of the fact that we cannot continuously increase the sensitivity of the test to detect syphilis, without diminishing its specificity and acquiring an increasing number of false-positive reports. The number of patients in whom the question of a false-positive reaction must be considered, is furnishing the biggest problem in the field of syphilis which confronts the clinician at the present time. I shall not attempt to review the various procedures which have been offered to differentiate between the socalled biologically false-positive tests, the false-positive tests which occur with other diseases and other conditions, and the positive test that occurs with syphilis. A new procedure which offers greater promise has been introduced within the past decade, and its study is furnishing us a new knowledge concerning the disease and the tests we have formerly used in its study.

Treponema pallida immobilization studies are exploring changes in the serum which are not dependent upon the development of reagin in the blood. The immobilizating antibodies which are developed in response to infection with Treponema pallida do not appear as early in the course of the infection as reagin. The tests, in their present stage of development, are compliacted and expensive to perform, and they will not replace the precipitation tests which are inexpensive and relatively simple. Currently, the greatest field of usefulness for these studies is in that group of patients who have persistently low titer positive serological reports, without clinical manifestations of the disease. In this restricted field they are furnishing a new tool which will afford the patient protection against an incorrect diagnosis, and they are affording the clinician valuable assistance in a difficult problem which will occur with increasing frequency as the standard tests for syphilis are applied to ever increasing numbers.

Any test which approaches 98% or 99% in its accuracy, must be accepted as a very good test, but an error of 1%, or even one-half of 1%, will yield an undesirable back-log of several thousand mistakes when it is applied to several millions of people. At the present time, serum tests for syphilis are performed on all members of the military service, on all of certain large groups of em-

ployees, on all of the patients who are admitted to many hospitals, on all blood donors, and on all members of certain communities at times, for epidemiological study. While the over-all object of such studies is good, and the detection of unknown infection is highly desirable, the immediate result of some individuals who may not have the disease is a calamity of first magnitude. For this reason, I feel that the investigation and attempt to differentiate between false-positive reports, and low titer reports which are significant, is highly desirable, and such studies should be encouraged. We should recall that the original complement fixation test was much more expensive, and a much more complicated procedure, than the standard precipitation tests which are so widely employed today.

We have reached now a point where the incidence of new infections is falling, and where the complications which we see in late syphilis are diminishing in number. It is too early, however, to celebrate the victory. Let us first consolidate our gains, and drop neither our educational program, nor our epidemiological studies, nor the investigations which are being carried out on a disease which still has many unanswered questions. The standard tests for syphilis may measure an important change in the blood serum of our patients which is not in any way related to syphilis. A positive test may indicate that the patient has recently been vaccinated, has infectious mononucleosis, or perhaps some occult disease whose clinical manifestations are not yet evident. These patients with low titer serological tests need study. Many of them do not have syphilis. They do not profit by treatment for syphilis. The psychological trauma of such a designation may do irreparable damage. Caution and consideration should replace the casual diagnosis of syphilis, supported by no evidence but a positive serologic test which measures the increase in a nonspecific substance, whose nature and composition is still far from completely understood.

FERMENTATION TESTS FOR IDENTIFICATION OF BRUCELLAE

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We have recently[2] confirmed earlier reports that brucellae attack several carbohydrates, and upon extending these observations we found that qualitative fermentation tests could be employed to distinguish species in the genus *Brucella*. Our success in developing these tests stemmed from the initial postulates that (1) a brucella fermentation medium must be low in available nitrogen in order to obviate deaminations and concomitant alkalinization of the medium and (2) must be sensitive to only slight glycolysis, and therefore (3) a relatively massive inoculum, to supply preformed enzymes, must be used.

These tests are convenient and reliable for routine identification of species in the genus *Brucella* and they serve as useful adjuncts to sensitivity tests for identification of atypical strains. However, the procedures for preparation of the fermentation mediums (aseptic addition of carbohydrate solution to heat sterilized basal medium) are not suitable for a routine diagnostic laboratory. Practical procedures for performing fermentation tests with brucellae, employing tableted mediums, have now been devised; the development, application, and reliability of these tests are the subject of the following communication.

Methods

Inoculums. Aqueous suspensions of brucellae were obtained from the 48 hour growth on supplemented Albimi brucella agar, as in our previous studies.[3] For routine tests, inoculums of suitable density were obtained by harvesting two slants with sufficient sterile distilled water to give *ca.* 0.8 ml. of bacterial suspension. The medium for each slant culture was 5 ml. of supplemented brucella agar in a 20 x 150 mm. screw cap tube.

Fermentation medium. Composition of the revised medium, in mg. per tablet, was: dipotassium phosphate, 1.74; agar, 5; phenol red, 0.02; yeast extract, 0.5; carbohydrate, 20; crystal violet, 0.002; and polyvinylpyrrolidone (PVP), 22.24. These revisions of our former medium,[2] discussed in detail below, were dictated by the desirability of making it applicable to tests on enteric bacilli[5] and by technical problems associated with dispensing biologicals in tablet form. The revised medium was pressed into 50 mg. tablets which, if not used for several weeks, were stored over anhydrous calcium chloride.

Test procedure. Deposit one tablet in a 12 or 13 mm. tube, add 1

With the technical assistance of Lorraine Scott and Jane Nakamoto. Aided by a grant from the Research Board, University of California.

ml. of distilled water, plug the tube with cotton, and steam 10 minutes to melt the agar and to destroy nonsporulating contaminants. Inoculate the dissolved medium with 0.1 ml. of bacterial suspension, incubate aerobically at 35-37 C., and read after 48 and 96 hours' incubation.

Results

Routine tests with representative strains. Employing our former experimental conditions,[2] and with inoculums of 10-30 billion cells, final readings of the fermentation tests could be made after one to three days' incubation with strains of *B. melitensis* and *B. suis*, and after two to four days' with *B. abortus*. Similar results have been obtained with tableted mediums (Table 1). For routine fermentation tests on brucellae, as indicated in Table 1, mannitol was included as a negative control and reference tube, and glucose as a positive control.

Table 1

Routine Fermentation Tests on Representative Strains of Brucellae

SPECIES	STRAINS POSITIVE/STRAINS TESTED					
	Mannitol	Glucose	Inositol	Maltose	Rhamnose	Trehalose
B. Abortus	0/30	30/30	30/30	0/30	30/30	0/30
B. Melitensis	0/13	13/13	0/13	0/13	0/13	0/13
B. Suis	0/41	41/41	0/41	41/41	0/41	41/41

Size of inoculum. Since there is considerable variation among strains of brucellae in respect to rate and abundance of growth, and since turbidimetric standardization of suspensions is a relatively laborious procedure, inoculums of *ca.* 1-900 x 10^8 organisms were examined in respect to their effect on fermentation tests. As judged from the nine strains examined (Table 2), an inoculum containing from 100-500 x 10^8 organisms should give reliable results. The tubes became too opaque for precise readings with inoculums greater than 750 x 10^8 organisms, and the incubation period was unduly prolonged with fewer than *ca.* 50 x 10^8 organisms. In this connection it may be noted that during the examination of 60 strains of brucellae by the routine procedure recommended above (i.e., using cells obtained by harvesting 2 slants with 0.8 ml. of sterile water), the inoculums always fell within the range of 100-300 x 10^8 organisms.

Contaminants. Evidence of contamination was sought in 479 tests by gross examination of the fermentation tubes and by single loop inoculations from these onto sectors of "purity" plates. Four contaminants were found, and three of these necessitated repetition of the tests. It appeared likely, however, that these were introduced with the inoculums since no contaminants appeared in 500 control (uninoculated) tubes of tableted fermenta-

tion mediums. During the initial examination of tableted mediums, tubes were steamed only 5 minutes before inoculation, and mold contaminants were common. No such contamination was found after the heating period was increased to 10 minutes. Heating an additional 10 minutes did not appear to alter the results obtained in subsequent fermentation tests, whereas a heating period of 30 minutes caused some decomposition of labile carbohydrates, particularly maltose.

Discussion

The fermentation patterns of 175 "typical" and "slightly atypical"[3] strains of brucellae have been determined during the course of this and previous[2] work, and in no instance were irregularities found. Hence either fermentation or dye sensitivity tests will establish the species for 85 to 90 per cent of strains which are encountered in the United States.[3] To be sure, the simplicity of sensitivity tests, particularly when dye tablets are employed,[4]

Table 2

Effect of Concentration Inoculum on Fermentation Tests With Brucellae

SPECIES	Days' Incubation	Inoculum x 10^{-6}	STRAINS POSITIVE/STRAINS TESTED				
			Glucose	Inositol	Maltose	Rhamnose	Trehalose
Brucella Abortus	2	875	2/2	0/2	0/2	0/2	0/2
		175	3/3	0/3	0/3	0/3	0/3
		35	2/3	0/3	0/3	0/3	0/3
	4	875	2/2	2/2	0/2	1/2	0/2
		175	3/3	3/3	0/3	3/3	0/3
		35	3/3	2/3	0/3	2/3	0/3
		7	3/3	0/3	0/3	0/3	0/3
	6	875	2/2	2/2	0/2	1/2	0/2
		175	3/3	3/3	0/3	3/3	0/3
		35	3/3	3/3	0/3	3/3	0/3
		7	3/3	0/3	0/3	0/3	0/3
Brucella Melitensis	1	875	2/2	0/2	0/2	0/2	0/2
		175	3/3	0/3	0/3	0/3	0/3
		35	2/3	0/3	0/3	0/3	0/3
	2	875	2/2	0/2	0/2	0/2	0/2
		175	3/3	0/3	0/3	0/3	0/3
		35	3/3	0/3	0/3	0/3	0/3
		7	3/3	0/3	0/3	0/3	0/3
	4	875	2/2	0/2	0/2	0/2	0/2
		175	3/3	0/3	0/3	0/3	0/3
		35	3/3	0/3	0/3	0/3	0/3
		7	3/3	0/3	0/3	0/3	0/3
Brucella Suis	1	875	2/2	0/2	2/2	0/2	0/2
		175	3/3	0/3	3/3	0/3	2/3
		35	3/3	0/3	2/3	0/3	1/3
	2	875	2/2	0/2	2/2	0/2	2/2
		175	3/3	0/3	3/3	0/3	3/3
		35	3/3	0/3	3/3	0/3	3/3
		7	3/3	0/3	0/3	0/3	0/3
	4	875	2/2	0/2	2/2	0/2	2/2
		175	2/2	0/2	2/2	0/2	2/2
		35	2/2	0/2	2/2	0/2	2/2
		7	3/3	0/3	1/3	0/3	0/3

makes it unlikely that this procedure will be discarded; however with frankly atypical strains, notably those which are acutely dye-sensitive, fermentation tests help materially in determining the species.[2] In addition, these fermentation tests have contributed to the characterization of abortus-melitensis and suis-melitensis intermediate strains.[1, 2]

Several revisions of our original fermentation medium were embodied in the tableted formulation. Both formulas give mediums containing 0.01 M phosphate. However the disodium phosphate of the former medium was replaced with the less hygroscopic dipotassium phosphate in the tableted medium. Monopotassium phosphate was omitted from the tableted medium since polyvinylpyrrolidone (PVP) is slightly acidic; both mediums, before inoculation, are frankly alkaline (pH 7.6 to 7.8) to the indicators. The change from 0.2 to 0.5 per cent agar was a matter of expediency; either concentration is suitable for fermentation tests with brucellae when phenol red is the pH indicator, but with the latter concentration the tableted mediums, when employed for fermentation tests on enteric bacilli, will detect the production of gas, as well as acid.[5] Similarly, yeast extract was added to the revised medium in order to make it more nearly universally applicable. The massive inoculums employed in brucella fermentation tests apparently provided available nitrogen for adaptive enzymes, but with micrococci and enteric bacilli addition of 0.05 per cent yeast extract promoted more rapid fermentation of disaccharides and, with salmonellae or coliforms, better gas production. The simplification in sterilization procedures required both a change in pH indicator and the addition of a bacteriostat. Cresol red, though a reliable and sensitive indicator in our former medium, tended to give false positive tests when carbohydrate was added to the basal medium before steam sterilization, whereas phenol red presented no such problem. Addition of crystal violet as a bacteriostat did not appear to affect fermentation tests on brucellae, or on gram positive nonsporulating bacteria,[5] whereas it did effectively prevent significant growth of sporulating bacteria.* Finally, several potential binders (dicalcium phosphate, calcium carbonate, powdered glass, gelatin, sodium and calcium salts of polymerized acrylic acid, methyl cellulose, carboxymethyl cellulose, and ethyl cellulose) for these tablets were examined. None equalled PVP in respect to neutrality, freedom from buffering action, stability, solubility, freedom from toxicity, metabolic inertness, and desirable physical characteristics.

Summary

Tableted mediums were developed for use in routine fermenta-

* In tubes inoculated with more than 500 x 10^6 cells, and which were incubated more than 8 days, contaminating **Bacillus** species did occasionally appear.

tion tests for identification of species in the genus *Brucella*. The tablets gave results comparable to those formerly obtained with less practical non-tableted mediums. They may be used as reliable alternatives to dye sensitivity tests and, with atypical strains of brucellae, provide valuable supplements to dye sensitivity and other biochemical tests.

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TABLETED MEDIUMS
For Biochemical Tests in Diagnostic Bacteriology

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The simplicity inherent in the fermentation tests developed for brucellae[3] convinced us that similar procedures should be even more applicable to other groups of bacteria. We were particularly impressed by the economy in time of preparation and storage space which are realized with tableted biochemical test mediums. Furthermore, use of large inoculums and inclusion of a bacteriostat in the tablets obviates some of the imperfections noted in other "rapid" biochemical tests.[1, 5]

In diagnostic bateriology, carbohydrate fermentation tests are usually in greatest demand, but urea, indol, sulfide, and motility tests are also frequently indicated. The following report therefore includes all these, their development into tableted substrates, and their use in the examination of bacteria which are associated with disease processes in man.

Experimental Methods

Formulation of mediums. The basal fermentation medium was developed with both brucellae and other bacteria as test organ-

isms, and along lines already discussed for the former.[3] The experimental approach was similar for development of additional mediums, the goal in each instance being to provide a medium which could be unitized into concentrated and stable tablet form, which would not require autoclave sterilization for use, and which, in a few hours, would give results comparable to those obtained after 24 hours' incubation with more conventional mediums and procedures. The final formulas for motility-sulfide, indol, and urea test mediums, thus developed, are given in Table 1. It may be noted that the indol tablet is similar to that devised by Hoyt[2] but employs a soluble binder, and the urea medium is patterned after that of Stuart, van Stratum and Rustigian.[4]

Table 1

Composition of Indol, Urea, and Motility-Sulfide Mediums (MG./ML.)

Indol:		**Motility-Sulfide:**	
Dipotassium Phosphate	2.44	Sodium Chloride	5.00
Monopotassium Phosphate	0.82	Potassium Chloride	2.50
Yeast Extract	0.50	Disodium Glycerophosphate	3.15
l-Tryptophane	0.50	Yeast Extract	1.00
Crystal Violet	0.002	Agar	3.00
Polyvinylpyrrolidone	45.74	Glucose	0.20
		Ferric Ammonium Citrate	0.20
Urea:		l-Cystine	0.10
Dipotassium Phosphate	11.60	Triphenyl Tetrazolium Chloride	0.05
Monopotassium Phosphate	9.28	Crystal Violet	0.001
Yeast Extract	0.10	Polyvinylpyrrolidone	9.80
Urea	20.00		
Phenol Red	0.02		
Crystal Violet	0.002		
Polyvinylpyrrolidone	9.00		

Inoculums. For routine tests, each bacterial strain to be examined was inoculated, from a slant or single colony, onto a 20 mm. slant (5 ml. of medium) and the growth was harvested after 16-24 hours' incubation with 1.0 ml. of sterile distilled water; 0.1 ml. of this suspension then served as inoculum for each biochemical test.

Test procedure. Save for the exceptions noted below, one tablet was deposited in a 12 or 13 mm. tube and 1 ml. of distilled water was added. The tube was plugged with cotton and steamed 10 min., after which 0.1 ml. of inoculum was added and mixed with the medium; the tube was then incubated aerobically at 35-37 C. Exceptions to this procedure were:

(1) because of the heat lability of urea, this medium was steamed only 5 min., and

(2) 2 ml. of distilled water was added to the motility-sulfide tablet, the tube was plugged, and the medium was steamed 10 min., thoroughly mixed, cooled, and inoculated by stabbing one loopful of bacterial suspension 3-5 mm. into the agar. Indol was detected by depositing 5 drops of Kovac's reagent in the test medium.

Results and Discussion

Variation in inoculums. The history and concentration of the inoculums were examined in some detail since it seemed important to establish the limits within which these could vary without unfavorably influencing results obtained in subsequent biochemical tests. The growth mediums from which inoculums were harvested were first compared and no significant differences were noted, the results being comparable with infusion agar (Difco), trypticase soy broth (BBL) solidified with 2 per cent agar, and Kligler's iron agar (BBL). Satisfactory results were also obtained when slants were harvested after only 6 hours', rather than 24 hours', incubation; hence it was possible and sometimes advantageous to inoculate a slant from a single colony early in the day, and later that day to proceed with several biochemical tests, the latter being read the following morning. Bacterial suspensions were usually used immediately after harvest but could also be used after having been maintained for some time at 4° C. For those biochemical tests listed in Table 2, e. g., no significant decrease in enzymatic activity was found with 6-day suspenpensions of *Corynebacterium xerose, Micrococcus pyogenes* var. *aureus, Escherichia coli, Proteus mirabilis, Salmonella typhimurium,* and *Shigella flexneri.*

Information concerning the limiting concentrations of inoculums within which satisfactory results can be obtained is presented in Table 2, which contains data covering four species. Cultures were harvested from 20 ml. of medium in 5 oz. bottles in order to obtain the concentrated suspensions required here, but the other experimental conditions were those already described. It is apparent from

Table 2

The Effect of Concentration of Inoculum on Biochemical Tests

SPECIES	Inoculum x 10^{-3}	Glucose	Maltose	Mannitol	Indol	Urea
Micrococcus Pyogenes Var. Aureus	300	2	2	24	—	4
	100	2	2	24	—	4
	10	24	24	24	—	24
	1	—	—	—	—	—
Escherichia Coli	300	2	2	2	2	—
	100	2	4	2	2	—
	10	2	24	4	2	—
	1	4	24	4	2	—
Salmonella Typhimurium	300	2	4	2	—	—
	100	2	6	2	—	—
	10	2	24	2	—	—
	1	4	24	4	—	—
Shigella Flexneri	300	2	—	2	2	—
	100	2	—	2	2	—
	10	2	—	4	2	—
	1	4	—	4	4	—

Readings were taken after 2, 4, 6, and 24 hours' incubation, 2=positive after 2 hours, 4=Positive after 4 hours, etc.; —=Negative after 24 hours.

this table that the enteric bacilli gave positive tests within 24 hours with as few as 1×10^8 organisms, and usually within 6 hours with inoculums containing $10\text{-}300 \times 10^8$ organisms.

Comparable results in respect to concentration of inoculums were obtained with *Aerobacter aerogenes, Proteus mirabilis, Proteus vulgaris, Salmonella paratyphi B, Salmonella thompson,* and *Shigella dysenteriae.* With inoculums outside the limits of $2\text{-}400 \times 10^8$ organisms, gas was not always obtained in fermentation tests on aerogenic species and false negative tests were obtained with inoculums containing less than 0.5×10^8 organisms. Finally, it may be noted that satisfactory results were obtained by transferring a loopful of cell paste (i.e., approximately 1 mm.^{3}) directly from the growth medium to each test medium, thus obviating the need for sterile water with which to prepare suspended inoculums.

Most species other than of enteric bacilli were enzymatically less active and the time-concentration factors were less satisfactory. Positive fermentation tests were obtained only after 24 hours' incubation with inoculums of 10×10^8 *Micrococcus pyogenes* var. *aureus* organisms (Table 2), and similar results were obtained with *Corynebacterium diphtheriae* and *C. xerose.* In routine tests, however, the inoculums were consistently within acceptable limits of concentration (Table 3).

Table 3

Representative Biochemical Tests With Tableted Mediums

SPECIES	Inoculum $\times 10^{-8}$	CARBOHYDRATES					Indol	Urea	Motility	Sulfide
		ACID				Gas				
		Glucose	Lactose	Mannitol	Sucrose					
Aerobacter Aerogenes	95	++	++	++	++	++	—	—	—	—
Alcaligenes Faecalis	90	—	—	—	—	—	—	—	—	—
Bacillus Anthracis	50	++	—	—	+	—	—	—	X	X
Brucella Bronchiseptica	55	—	—	—	—	—	—	++	++	—
Corynebacterium Diphtheriae	30	++	—	—	—	—	—	—	X	X
Corynebacterium Ulcerans	125	++	—	—	—	—	—	++	X	X
Listeria Monocytogenes	20	++	—	—	++	—	—	—	X	X
Micrococcus Pyogenes Var. Albus	125	++	+	—	++	—	—	++	X	X
Neisseria Catarrhalis	20	—	—	—	—	—	—	—	—	—
Neisseria Meningitidis	90	++	—	—	—	—	—	—	—	—
Paracolobactrum Species (Bethesda Group)	50	++	—	++	—	++	—	—	++	+
Pasteurella Multocida	10	+	—	—	+	—	++	—	—	—
Proteus Rettgeri	90	++	—	+	++	—	++	+	++	—
Pseudomonas Aeruginosa	105	++	—	+	—	—	—	—	++	—
Salmonella Montevideo	60	++	—	++	—	++	—	—	++	++
Salmonella Paratyphi A	85	++	—	++	—	++	—	—	++	—
Shigella Dysenteriae	45	++	—	—	—	—	—	—	—	—
Shigella Sonnei	80	++	—	++	—	—	—	—	—	—

++=Positive after 6 hours, +=Positive after 24 hours, —=Negative after 24 hours incubation; X—not done.

Examination of representative species. Following the routine test procedures recommended above, 86 stock and recently isolated cultures were examined in order to establish the validity of tableted mediums for use in diagnostic bacteriology. The results confirmed

the reliability of these procedures for examination of many of the commonly encountered pathogens, as shown in Table 3, which contains data covering 18 representative strains. These procedures did not give satisfactory results, however, with species such as the pneumococcus which present only scant growth on solid mediums.

The results obtained with two groups of organisms bear additional comment. Those with *Pseudomonas aeruginosa* were of particular interest since, like the brucellae, it commonly gives weak or negative fermentation tests by conventional procedures, and therefore may be confused with nonfermentative species. Twenty cultures received under the title of "pseudomonas" and recently isolated from clinical specimens were examined. Four of these gave tests quite different from those shown in Table 3 and were found upon further scrutiny not to be *Pseudomonas* species. Of the 16 authentic strains, 7 produced little or no pigment on infusion agar, and only 1 gave a positive fermentation test in a medium containing 1 per cent tryptose (Difco), 0.5 per cent glucose, and 1 mg. per cent brom cresol purple. However, with the tableted mediums all were glucose and xylose positive, 15 were mannitol positive, 15 were motile, and all were lactose, salicin, indol, and sulfide negative.

Finally, 9 strains of paracolon bacilli were examined with tableted mediums. Seven were negative in lactose after 24 hours' incubation, but after 6 days' (or less) incubation the results from lactose, sucrose, salicin, indol, and motility tests permitted exclusion of salmonellae and shigellae. This brief survey suggests, therefore, that tableted mediums will detect paracolon bacilli as readily as do conventional biochemical tests.

Summary

Mediums for fermentation, indol, urea, motility, and sulfide tests were prepared in tablet form. Compared with conventional prepared mediums, the tablets are appreciably more stable and require a minimum of storage space. They permit preparation of mediums as needed, within a few minutes, and without autoclave sterilization.

A variety of organisms was examined with the tableted mediums. The results, qualitatively, were compatible with the species and, quantatively, compared favorably with those obtained by other "rapid" biochemical test procedures.

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HISTOLOGICAL OBSERVATIONS OF THE KIDNEY AND LIVER IN SCORBUTIC GUINEA PIGS*

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History and Introduction

Scurvy was recognized as a disease of great importance in the writings of Hippocrates and Pliny. Straby described the disease in his writings concerned with the armies of Caesar Germanicus and Aelius Gallus. Although the disease has been considered for centuries, it was not until Lind in the eighteenth century associated scurvy with a dietary deficiency, mainly the lack of citrus fruit juices. The first suggestion that scurvy was an avitaminoses was made by Funk; however, the first research evidence was initiated by Holst and Frohlick (1907) when they produced scurvy experimentally in guinea pigs. In 1932, Waugh and King precipitated anti scorbutic factor which appeared identical to the hexuronic acid previously isolated by Szent-Gyorgyi. It was not long afterwards in 1933 that Reichstein and Haworth announced the first successful synthesis of ascorbic acid.

Even though the etiology of scurvy is known, there's much to be learned about the basic function of ascorbic acid in the cell. It is generally considered that one function of vitamin C in the body is that of a hydrogen transporter in cellular respiration. Another experimentally well-founded function is the participation, in a still unknown manner, in the formation of colloidal intercellular substances which comprise those of cartilage, dentine, and the material of bone and, furthermore, the collagen of all fibrous tissues and non-epithelial cement substances. In addition ascorbic acid has been linked with amino-acid metabolism, it has been shown in vitro that dehydro-ascorbic acid dehydrates, amino acids, such as leucine, with the formation of ammonia and strongly reducing acidic compounds. Also it has been shown that the capacity for metabolizing carbohydrate is moderately lowered in scurvy.

Vitamin C appears to be important in several phenomenona of particuar interest, for instance, beneficial effects of large doses of this vitamin may act as a detoxicant for toxic drugs, chemicals and toxins of infectious organisms. Actually in a survey of the literature, one is amazed at the tremendous number of papers concerned with different therapeutic uses of this vitamin.

We might think that today with the available knowledge of basic nutrition there would not be a scurvy problem. However,

* Read before ASMT convention, June, 1954, Miami Beach, Florida.

this is not true. For instance, when the bones of all children coming to autopsy in the John Hopkins Hospital were studied during more than a decade, unmistakable evidence of scurvy was found in 11.7 percent of 487 children between ages of 3 and 19 months. It is not always a malnourished child that develops avitaminoses; many well-fed, healthy appearing youngsters manifest signs of scurvy and it is very likely that many subclinical cases exist.

Since scurvy remains a problem and since the function of ascorbic action within the cell is questionable it appeared worthwhile to approach the problem using some of the recent histological technics. Our approach was patterned after the work of Kuntz and Sulkin in which they demonstrated the ascorbic acid content of the autonomic neurones changed under various pathologic conditions.

Material and Methods

Guinea pigs, because of their inability to synthesize sufficient amounts of ascorbic acid for their body needs, were used as the experimental animal. The guinea pigs were fed a vitamin C free diet; made so by grinding and heating Purina rabbit pellets. To this ground mixture, brewers yeast in the amount of 5 percent was added. This mixture was fed to both the control and experimental animals. The diet of the control animals was supplemented by the addition of ascorbic acid to their drinking water. The experimental animals were left on the scorbutic regime until the animal was no longer ambulatory; at which time experimental and control animals were killed and tisues were removed for examination. Samples of kidney and liver were placed in the fixative necessary to demonstrate the particular substance or activity that was under scrutiny.

Ascorbic acid was localized by fixation and staining in an acidified silver nitrate solution (Barnett and Fischer 1943). The desoxyrebonucleic acid was demonstrated by means of the Feulgen method utilizing Schiff's reagent (Lillie 1948). A counterstain of fastgreen was used in most instances; although we had better preparations without it. Tissues fixed in 10 percent formalin were stained with 0.25 percent toluidine blue for 30 minutes, no counterstain, for the demonstration of ribonucleic acid (Sulkin 1951).

Glycogen and mucopolysaccharides were studied after the action of periodic acid followed by treatment with Schiff's reagent (Lillie et al 1947). The glycogen was differentiated from mucopolysaccharides by treatment with a buffered digestion mixture of 1 percent malt diastase for one hour at 37° C.

The lipids were demonstrated in tissue fixed in 10 percent formalin or a formalin-cadmium-calcium fixative (Baker 1944).

Frozen sections were made and stained with Sudan black in 70 percent ethanol. For the study of acid phosphatase the tissue was fixed in cold acetone and incubated in a buffered substrate of sodium glycerophosphate at a pH of 4.65. The alkaline phosphatase was fixed in cold (O°C) 80 percent ethanol and incubated (37°C) for 2 hours in sodium glycerophosphate buffered at pH 9.0 (Gomori 1939 as modified by Kabat et al 1941).

The Golgi apparatus was demonstrated by placing the tissue in Champy's fixative for 24 hours and in 2 percent osmic acid solution for 6 days at 37 degrees C. Mitochondria were demonstrated in sections which had been fixed by Regaud's method and stained with hematoxylin (Fain and Wolf 1944).

Besides the special stains, representative samples of the tissues were routinely stained by the Mallory's phloxine-methylene blue method.

Results and Discussion

The ascorbic acid content of the liver and kidney was greatly diminished in the scorbutic state. Few cells contained demonstrable amounts of this vitamin. A correlative decrease was observed in the phosphatase activity. The amount of P.A.S. (Periodic Acid Schiff) positive substance was less in the extremely scorbutic animals due to decreased quantities of glycogen in the cells.

The Golgi complex appeared normal in most cells, although dispersed in a few cells. The mitochondria in nearly all of the cells appeared enlarged and swollen; many were clumped and coalesced. Since the mitochondria are commonly considered a center of fat metabolism, it might be assumed that some alteration in the fat content of the cells might occur. This was the case in a few cells, although the increased Sudanophilia was inconsistent.

Many observers consider vitamin C activity to take place in either the Golgi complex or the mitochondria. Others believe the activity is in both of the organoids. Our observations do not substantiate any special correlation.

Although histological alterations were noted in both the liver and kidney, it does not appear from this preliminary study that these organs are greatly altered by scurvy.

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THE LABORATORY ROLE IN Rh SENSITIZATION

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With the advance in knowledge of the Rh factor and Rh incompatabilities there have arisen many new aspects of interest to the medical technologist. There is a growing concern in the significance of our antibody titres, the serum bilirubin, the erythroblast count, and the hemoglobin of the child. The laboratory plays an important part by running the routine titre tests on all Rh negative mothers and by performing crossmatches that are completely compatible in the A B O and Rh system, thus lessening the chance of artificial sensitization by transfusion.

From the viewpoint of the obstetrician the Rh factor is probably of prime concern. It is obvious that prophylaxis of Rh sensitization is so important that it cannot be over emphasized. The present important duty is to prevent Rh negative females regardless of age or marital status from receiving Rh positive blood. The Rh-Hr status of every mother should be determined. If she has had previous transfusions the Rh-Hr status of the donors should also be obtained if possible. Antibody tests should be done as early in pregnancy as posible. If they are negative they may be repeated in thirty-four weeks, however, if they are positive they should be repeated at intervals of four weeks or oftener.

The interpretation of the tests for antibodies is also quite important. If they remain negative by the thirty-fourth week the prognosis is good in regard to iso-immunization. If they appear in twelve weeks or less they usually indicate the possibility of a mild hemolytic disease. As yet there is no accurate correlation between the antibody titre and the severity of the hemolytic disease. At present there are no means available of neutralizing in vivo of the Rh-Hr antibodies in pregnant women.

There are three separate and distinct types of Rh antibodies which should be tested for: Saline agglutinins, detectable when a physiological solution of sodium chloride is used as a diluent for serum and liquid for suspension of red blood cells; Serum-albumin agglutinins known as conglutinins, not detectable in saline but manifested when human serum or bovine albumin is used as a diluent; incomplete or blocking antibodies which attach themselves to the red cells but do not produce clumping and are detected by means of an indicator. It is believed that the blocking antibodies have a much graver prognosis than the saline agglutinins. For the greatest benefit to the doctor, tests should include all three of these.

If the patient has had a previous history of blood transfusions, Rh positive blood should never be used. The possibility of a fatal blood transfusion reaction cannot be overemphasized.

At birth after these routine tests of Rh, Coombs, erythroblast count, and bilirubin have been done and are found to be positive, an exchange transfusion should take place, thereby helping to eliminate one of the most important complications of erythroblastosis fetalis which is brain damage and commonly known as kernicterus. Fifteen per cent of liveborn infants with erythroblastosis are affected by it. About 70% of the babies with this die within seven days after birth, and many succumb during the first year of infectious diseases. Thus it is one of the most important causes of post-natal death in the erythroblastolic infant. Kernicterus has been proved to be preventable by exchange transfusions and the treatment of kernicterus has become the most important consideration in the treatment of erythroblastosis fetalis, indicating thereby the importance of pre-natal investigation of Rh negative women so advance preparation can be made for treatment of the baby.

The brain damage is not present at birth, but usually manifests itself late in the second day. It does not occur in the absence of considerable jaundice. Kernicterus is likely to occur in babies whose serum bilirubin level is above 30 mg./100 cc. and unlikely to occur when the level of bilirubin remains below 20mg./100cc. Therefore, even though it has not been proved that bilirubin is the actual cause of brain damage, tests of serum bilirubin in babies with erythroblastosis serves as an extremely valuable guide to treatment.

By the exchange transfusion, repeated if necessary, the serum bilirubin can be kept at relatively low levels and the lessening of the probability of kernicterus will result.

The only thing that is accomplished by these tests is it has alerted the doctor so that he can be ready to rescue the baby with a transfusion immediately after birth. This means immediate preparation will be made of the baby's cord blood for blood group, Rh reaction, and blocking antibodies as revealed by the Coombs technique.

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SPINAL FLUID CULTURES*†

JEWELL M. MITCHELL, B.S., M.T. (ASCP)

The primary aim of the Bacteriology laboratory should be to get the report back to the clinician as soon as possible. In the bacteriological examination of spinal fluids direct smears are of the utmost importance and will often permit a presumptive diagnosis. After the specimen is set up as a routine the remainder should be centrifuged at 2500 R.P.M. for 10 minutes and then a gram or methylene blue stain made. We prefer the gram stain at our laboratory and always use controls of a known positive cocci and known negative bacilli as a gauge of our staining technique.

Our routine procedure consists of the following: a gram stain is made and 1 cc. of spinal fluid is inoculated to a thioglycollate broth, 1 cc. to a tryptose phosphate broth and 1 loopful is placed on a chocolate agar plate and streaked. All chocolate agar plates are placed in a CO_2 jar. On all specimens from children a staphylococcus streak is made across the plate. It is very important that the chocolate plate be warmed to body temperature before inoculating. The thioglycollate broth is a very good medium for both aerobic and anaerobic growth and if the spinal fluid quantity is insufficient the thioglycollate broth is always favored and the tryptose broth simply omitted. The staphylococcus streak is added to the chocolate agar plates on spinal fluids from children because of the factors required for growth of hemophilus influenzae. It is not added to the spinal fluid cultures from adults since hemophilus influenzae is the cause of purulent meningitis in children, rarely in adults. The routine culture is incubated for 16 to 24 hours and read. If there is no growth at the end of 48 hours they are reported out. The organisms you are most likely to isolate from a spinal fluid are:

Neisseria meningitidis (N. intracellularis)
Pneumococci
Hemophilus influenzae
Staphylococci
Beta hemolytic streptococci
Bacteroides
Pseudomonas aeruginosa
Torula
Gonococci
Friedlander's bacillus
Mycobacterium tuberculosis

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* Presented at the G. S. M. T. Convention, Columbus, Ga., April, 1954.
† Reprinted by permission from the Georgia News Journal of the G. S. M. T., Oct., 1954.

In suspected meningitis, examination must be done at once because of rapid autolysis. There are a number of organisms which can cause meningitis. In meningitis patients, after you have determined the gram reaction and whether it is a cocci or bacilli, you can go a step further and either employ the oxydase and agglutination tests for the Neisserian group or the Neufeld Quellung reaction for the hemophilus or pneumococci. Many times these steps can mean the difference in 10 minutes and 48 hours in your report.

It is rather difficult to detect the presence of Neisseria meningitidis on a smear due to the debris and cells always present. The organisms are small gram-negative diplococci with the adjacent ends flattened. One can readily see the importance in fixing a control of a known positive cocci and known negative bacilli on each smear to be stained. For instance, in the case of diplococci, it is important to know as quickly as possible if it is pneumococci or a Neisseria. Of course the morphology will help to a great extent in differentiation.

In direct smears the pneumococci are lancet-shaped gram-positive diplococci. Blood agar is the medium of choice with the bacterial colony appearing on the plate within 24 hours as a small colony surrounded by a zone of green hemolysis. The pneumococcus is identified by serological reactions, chiefly the Neufeld Quellung reaction. Type specific immune rabbit serums used in approximation with organisms and methylene blue. From experience I have observed that when antigen and antibody approximate each other a maximum reaction occurs (thus using equal parts of organisms with antisera.) All of you are probably aware of this fact as it is usually applied in serologic tests.

It should be noted that no sure distinction between the meningococcus and the gonococcus can be made on morphological grounds, and the identification of gonococci in gonococcal meningitis is dependent upon culture and differential fermentations. In isolating the meningococcus it is essential that the culture medium be warm when inoculated and kept warm until finally placed in the incubator. Growth is favored, especially in the primary isolation by incubation in an atmosphere containing 10 percent carbon dioxide. The fermentation of maltose serves to distinguish the meningococcus from the gonococcus. We have a crutch to remember the fermentations of dextrose, maltose and sucrose for the differentiation of the Neisseria. 210 machine gun corps. 2, 1, and 0 signifying the number of sugars fermented and m, g, and c signifying the organism. Gonococcal meningitis occurs, perhaps more frequently than formerly thought.

It is also difficult to detect hemophilus influenzae in direct smears from the centrifuged spinal fluid. When gram-negative bacilli are seen on direct smears from the spinal fluid they may

not necessarily be hemophilus influenzae. The possibility of Bacteroides as well as coliform bacilli must be considered. Hemophilus organisms are minute, gram-negative rods, sometimes thread-forming and pleomorphic. They are one of the most fastidious of the bacteria and require the presence of blood in the culture medium and in general requiring blood serum, ascitic fluid or certain growth accessory substances. Six types (A-F) of Hemophilus influenzae are recognized on the basis of precipitation of immune serum by capsular substance. Strains from cerebro-spinal fluid are usually of Type B. Two substances are necessary for the growth of this bacterium. One designated as the "X" factor is heat-stabile and associated with hemoglobin and the other, the "V" factor, is heat-labile. The satellite phenomenon, characteristic of the bacilli, is due to the formation of the "V" factor by other bacteria and its diffusion into the medium from the colony. The "X" factor is replaceable by hemoglobin or hematin. "Influenzal meningitis" is usually fatal.

If Mycobacterium tuberculosis is suspected acid-fast-stained smears of the centrifuged sediment should be studied. Prolonged centrifugation for 12 to 24 hours may increase the percentage of positive smears. As a general rule, a typical pellicle forms in spinal fluids from cases of tuberculosis meningitis when the spinal fluid is allowed to remain undisturbed at room temperature for periods varying from 1 to 18 hours. A prolonged and careful search should be made of smears since very few organisms are ever present. (100,000/cc. to be found in direct smear). If organisms are present, they will appear as fairly long, slender, irregularly stained, rather pleomorphic, acid-fast bacilli.

Bacteriodes should be suspected if gram-negative bacilli are found only in the thioglycollate broth medium. These strict anaerobic gram-negative bacilli have been isolated from cases of meningitis following chronic otitis media. The isolation of Bacteriodes requires special effort and patience.

Friedlander's bacillus is rare as the causative organism of meningitis.

Stained smears show gram-negative rods surrounded by a large capsule. Typing sera, when available, allow specific diagnosis.

Pseudomonas aeruginosa is a gram-negative motile bacilli, non-encapsulated and nonsporeforming. It grows readily on all culture media. Meningitis, due to this organism, has been observed to follow a lumbar puncture or operative exposure of the meninges.

I believe all of you are familiar with the morphology of the Staphylococci and Streptococci so I will only mention that meningitis can be due to these organisms but it is uncommon.

Cryptococcus neoformans is a yeast-like non-sporulating, non-mycelial, budding fungus characterized by the development of a

wide capsule in tissue and in culture. It has a marked predilection for the central nervous system and produces a subacute or chronic infection of the meninges (Torula meningitis).

Below you will find several facts concerning different medias and tests which I have mentioned and I hope it will provide additional information for you.

Differentiation of Members of Influenza-Bacillus group. Requirements for diagnosis:

1. Gram-negative, aerobic, non-spore bearing, nonmotile bacilli requiring hemin, coenzyme or both for growth.

2. X factor medium. Equal parts of "Levinthal stock" (used in Levinthal agar), autoclaved 15 minutes at 20 pounds pressure, and melted Proteose Agar No. 3 (Difco) (45 gms. per liter plus Bacto Agar 15 G. per liter). X factor is stable and can withstand this treatment; V factor is destroyed.

3. V factor medium. One part yeast extract to 9 parts of melted proteose No. 3 Agar (Difco). A method modified from Thiotta and Avery (1921).

4. H. influenzae-classifiable into 6 specific types by the polysaccharide elaborated.

5. Oxydase test for the Neisserian Group.

6. A 1 per cent solution of para-aminodimethylanalinemonohydrochloride used. The reagent should be prepared fifteen minutes in advance. A few drops are placed on suspicious areas of the plate. If organisms producing oxydase are present, the colonies will become pink, changing to red and finally to black.

7. Tryptose Phosphate Broth.

It is a liquid medium prepared without an infusion of meat, recommended for the cultivation of streptococci, pneumococci, meningococci, and other organisms generally considered difficult to cultivate.

8. Thioglycollate Medium.

A fluid medium which will support the growth of both faculative and strict anaerobes. The sodium thioglycollate acts as a reducing agent and good anaerobiosis is produced in all but the very upper portion of the medium. The formula includes methylene blue which acts as an indicator of anaerobiosis, the anaerobic portion of the medium being colorless, due to the reduction of the dye by the sodium thioglycollate, and the surface, aerobic layer of the medium being green. The medium naturally contains neutralizing substances which will prevent the inhibition of bacterial growth by any sulfonamide which may be present in the original inoculum.

"Attitude Training Program"[1]

ARE ATTITUDES IMPORTANT?

In our changing world, attitudes are more important than skills. The skills we learn today may be of little use ten years hence. The weapons we are now producing in such vast volume may be lying in rubbish heaps after this spurt is over. But attitudes are permanently effective. Training in character fits individuals and nations to handle any kind of power from the human fist to atomic fission.[2]

It has been said that there are two aspects of a hospital's care to its patients.

The first, and more important, is the technical skills which are used in the diagnosis and treatment of the sickness and injuries of patients. However, a patient can't evaluate the quality of these technical skills.

The other, the way in which hospital workers deal with the patient, or our "attitude" toward him, he can and does *evaluate*. In fact, the chairman of the Board of Trustees of a large teaching hospital has said that confidence and goodwill of patients and their guests depends to a greater extent on our attitude than on the more important technical skills!

It is important for hospital employees to be sensitive to the feelings of patients, in addition to caring for their physical needs, because:

1. A patient's mental condition may affect his recovery from a physical ailment.
2. Satisfied patients are the best public relations for the hospital.
3. The Rhode Island Hospital is endowed as a charitable institution, morally bound to dispense kindness as well as medical care.
4. The attitudes of employees toward patients is largely responsible for setting social tone or atmosphere in which patients will be hospitalized and in which employees will work. A pleasant tone attracts patients, doctors, and employees alike.
5. Sensitive treatment will win the confidence and good will of patients. Trustful patients will cooperate more willingly with employees during hospital interviews and procedures.

This article was sent to the N.D.S.M.T. Newsletter by Sister Moira, M.T., from Richardton Hospital, Richardton, North Dakota.

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THE GAVEL

In this, my last message as your president to the members of ASMT, I want to pass on one more thought for contemplation—"I am not concerned that I am not known, I seek to be worthy to be known." The old cry is heard constantly that Medical Technologists are not "known," that financial and personal gains are not rewarding enough, that professional recognition is not afforded, that special consideration is not given. We must accept and recognize our responsibilities; we must be individually ready and able by constant education, since interest, unfailing energy, constructive attitudes, scientific curiosity, and complete integrity to be worthy of the faith and the trust we seek. By honesty, industry, and unselfishness in service to others we will not be found wanting.

A great man once said that faithfulness and sincerity are to be held as first principles. Truly this can be said of the officers and committee members who served ASMT so energetically and willingly this past year. I am grateful to have this opportunity to thank all those who helped to make many ideas and thoughts become realities as well as to accomplish the daily tasks that make an organization vital.

It has been an honor and a privilege to serve you this past year and now I take leave of office with a sincere expression of best wishes to ASMT for continuing growth and accomplishments in the years to come.

RUTH HOVDE

EDITORIAL

THE CODE BY WHICH I PRACTICE MEDICAL TECHNOLOGY*

I am a Medical Technologist, a thinking individual, able to work with my mind as well as with my hands. There is compensation in my work. In being a part of that profession whose end is the mental and physical well being of mankind, my share lies in the performance of those technics whose results the physician will follow through and correlate with his clinical findings.

Whether I plan to practice the profession of medical technology for one year or for the remainder of the time I am physically and mentally able, I shall use the same meticulous care in the performance of the tests as I would if upon the results depended the well being of a member of my own family.

As a medical technologist it is not my purpose nor function to render a diagnosis, but to foster co-operation with the physician to whom laboratory data is indispensable.

As a medical technologist I am proud, with a pride that is tinctured with a true humility, with a pride in being one of the trio in the medical profession, the physician, the nurse, and the medical technologist, each of whom functions in his distinct way.

As a medical technologist, I am independent, with a co-operative spirit in working with my fellows in the medical profession. I do not want to encroach upon any one else's premises.

May I look upon my profession with the respect it merits, and so win the respect of those in related professions and the laity alike.

R. M.

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* Reprinted by request from the A.J.M.T. Vol. 13, No. 1, Jan. 1947.

DRAGONTEK AND THE NIGHT CALL

This is the second of the Dragontek series taken from "Annals of Unsung Heroines," Vol. O, No. 2, and tells of the trials of the technologist as he attempts to serve science.

The story you are about to read is true. Only the final diagnosis has been withheld to give you something to think about.

You're a technologist. Your name is Wednesday, the boss' name is Thursday. You're assigned to hematology. On the night of February 29, you're covering the night watch ("taking call" to you) and you retire about 10:30. At 11:30 you are awakened to the musical sound of the telephone and you learn there is a stat CBC. You cheerfully (?) go to the lab and get the count box and go to pediatrics where you pick up the slip. From it you learn the patient's name is Billie Vard; he is 10 years old and the chief complaint is abdominal pain.

You take the count and you notice the patient is very pale and the hemoglobin looks like it could do with a boost. There are two adults there speaking some foreign language—you think Italian. You don't understand Italian and you're not really interested, so you take the count and return to the lab.

Twelve midnight—you're back in the lab with the count trying to look scientific. The hgb you read as 4.6 gm. so you take a quick look at the red count. It's low. Upon careful counting you find the RBC to be 2.76 million, and the WBC to be 11.050. The differential count shows 68% polys, 23% lymphs, 2% monos, with 7% nucleated RBC. You rub your eyes again and note the RBC on the smear appear to have all the osises known to hematologists (microcytosis, anisocytosis, and poikilocytosis) with hypochromia, basophilic strippling and polychromasia thrown in for good measure. By this time you are not sure what you are seeing, but you report all this to thoroughly confuse the good doctor and give him the general impression this child has some kind of blood dyscrasia.

One a.m. you again retire and wonder for a few minutes between waking and sleeping what the diagnosis could be.

Within the next two days the suspect (blood and patient) is run through a veritable gamut of laboratory procedures. Various notables are taken to the hematology department to view the slides, but no positive identification can be made. It is generally agreed, however, that there are a few target cells and some elliptocytes.

March 3 you are requested to do the same blood tests on the patient's mother, and these results, plus the laboratory and x-ray findings on the patient, make the diagnosis.

Additional Laboratory Findings on Patient

Reticulocyte count	23.4%
Fetal Hgb.	1.6%
Target cells	few
Icterus Index	10 units
Osmotic Fragility	0.38-0.24 (Normal range 0.48-0.34)
Serum albumin	4.2 gm%
Serum globulin	3.0 gm%
Cephalin flocculation	2+
BSP retention (45 min)	2%

Laboratory Findings on Mother

RBC	5.34 million	Hgh	14.7 gm.
Hematocrit	47 vol.%		
Osmotic fragility			0.38-0.28
Target cells			few

The moral of the story is: always open your eyes when you are called at night to aid science. Who knows what may parade before you on the blood smear.

For the solution of this mystery, see page 189.

ABSTRACTS

AN IMPROVED METHOD FOR SIMULTANEOUS DETERMINATION OF EXCHANGEABLE BODY SODIUM AND POTASSIUM

C. V. Robinson, Walter L. Arans, and A. K. Solomon. (Harvard Medical School).
J. of Clin. Invest. 34, 134-40 (1955).

A differential counting technique is outlined for the simultaneous determination of exchangeable body sodium and potassium. The individual radioactivities of Na^{24} and K^{42} are measured without separating them.

RADIOACTIVE ISOTOPES AS AIDS IN MEDICAL DIAGNOSIS

Edith H. Quimby. (Columbia University College of Physicians and Surgeons.) New Engl. J. Med., 252, 1-9 (1955).

Radioactive isotopes are handled in the same manner by physiologic and metabolic processes, and have the same chemical reactions as their chemically identical counterparts but differ from them by the property of their atoms to disintegrate and emit radiation which makes it possible to follow them.

Their rate of disintegration is expressed by a statement of their *half lives*, i.e., the period in which half of all the atoms present in a particular isotope will have disintegrated, in the next half period half the remainder will disintegrate and so on. This half-life period varies with the isotopes from fractions of a second to millions of years. In medicine, for the most part, half lives vary between a few hours and a few weeks; radioactive potassium (K^{42}) has a half-life of 12.4 hours—its period for reduction to 1% is about 3.5 days; radioactive phosphorus (P^{32}) has a half life of 14.3 days—its reduction to 1% is over three months.

Radioactive isotope amounts are expressed in terms of curies, millicuries or microcuries. The microcurie of an isotope which is the normal one for diagnostic procedures is that quantity of an isotope in which 37,000 atoms disintegrate per second. An isotope with a long half life, a microcurie of it will have to contain more atoms that if it has a short one, to keep up the supply for disintegration.

Radioactive iodine (I^{131}) with a half life of 8 days, contains about 37,000,000,000 atoms per microcurie (contained in a very small part of a microgram—about one one-hundred-thousandth).

The radiation emitted may be beta or gamma, or both. Some radioactive isotopes are obtained in such a form that every atom of the element is radioactive and is said to be "carrier free." I^{131} is one of these. Others can be obtained only as a mixture with stable isotopes and are said to be "with carrier." Radioactive sodium (Na^{24}) is supplied with a specific activity of 90 mc. per gram of sodium. In a dose of this material there would be a million times as much stable as radioactive sodium.

The beta rays may be so unpenetrating that a small fraction of a millimeter of tissue will stop them. Two other limitations which exist in tests with radioactive isotopes are: the avoidance of chemical effect and avoidance of radiation effect. With carrier present the physiologic situation may be altered, for example, studies with radioactive iodine in the thyroid gland. Normally about a third of the dose of carrier-free radioactive iodine will concentrate in the thyroid gland within 24 hours. But if a few milligrams of stable iodine are used as carrier the uptake is appreciably lowered.

In medical procedures radioactive isotopes are used for dilution studies in determinations of the volume of blood, plasma, red cells and extracellular fluid. Radioactive iodine, used as a tag or label for human serum albumin, is used most frequently for blood-volume studies. The procedure may be

Procedure	Blood Volume
50 microcuries I^{131} injected in 5 ml. of saline solution	
Counts/microcurie/min.	30,000
Counts for 50 microcuries would be	1,500,000
After 5 minutes blood taken	
Counts for 5 ml. whole blood	1,500
For 1 ml. counts would be	
Dilution factor	
$\frac{1,500,000}{300} = 5000$ indicating 5000 ml. of whole blood	
With hematocrit of 0.45:	
Red cell volume	2,250 ml.
Plasma volume	2,750 ml.

Other uses for radioactive isotopes in medical procedures are: the rate of transfer from one part of the vascular system to another in order to ascertain some fact about the circulation; the rate of disappearance of an isotope that has been injected directly into a certain tissue to reveal the state of the local circulation in that tissue; and the concentration of the isotope in a particular organ or tissue, to determine something about the local function of that tissue.

A-B HEMOLYTIC DISEASE OF THE NEW BORN. ANALYSIS OF 1480 CORD BLOOD SPECIMENS, WITH SPECIAL REFERENCE TO THE DIRECT ANTIGLOBULIN TEST AND TO THE GROUP O MOTHER

Richard E. Rosenfield. (Mount Sinai Hospital and Dept. of Health, New York.) Blood, 10, 17-28 (1955).

The author has made a study of the role of the A and B factors as a cause of a disease similar to the Rh hemolytic disease of the new born. Oxalated umbilical vein blood was examined from every infant born during a nine month period at Mt. Sinai Hospital. Maternal blood was also studied. Where possible these tests were made: (1) group and Rh, (2) direct antiglobulin test, (3) hemoglobin, (4) reticulocyte count and red cell morphology, (5) plasma bilirubin and (6) osmotic fragility in 0.52 per cent sodium chloride solution.

The blood groups of the infants were classified as compatible or incompatible with those of the mothers. A third class which showed a positive direct antiglobulin test were analyzed separately for comparison. Eleven per cent of the group incompatible infants showed this reaction but none was found in the group compatible infants. These incompatible infants also showed that their mean hemoglobin was low, their mean reticulocyte count was high as also their mean bilirubin when compared with the group compatible infants.

Thirty-eight of thirty-nine mothers of these incompatible infants with positive direct antiglobin test were group O.

Increased osmotic fragility in hypotonic sodium chloride solution was found in thirty-one out of the thirty-eight infants with positive direct antiglobulin test.

MECHANISMS CONTROLLING CATION CONCENTRATIONS IN THE HUMAN CELL; EVIDENCE FROM THE EFFECT OF IODOACETATE ON Na AND K EXCHANGE RATES OF THE ERYTHROCYTES

W. D. Love, J. A. Cronvick, and G. E. Burch. Tulane University and Charity Hospital of Louisiana. J. of Clin. Invest., 34, 61-6 (1955).

Because of the abnormalities of intracellular electrolyte distribution occuring in a variety of illnesses the mechanisms controlling intracellular electrolyte content are of clinical interest. An *in vitro* test under simulated physiologic conditions was made with human whole blood and iodoacetate which is known to abolish glycolysis and selective cation accumulation in erythrocytes. The changes in the rates with which Na and K entered and left the RBC was measured by using Na^{22}, K^{42}, and Rb^{86}. The calculations of exchange rate were made by the method of Sheppard and Martin. There was a net flow of sodium into the cells (1.18 mEq Na per L of RBC per hour) and a greater flow of potassium out of them (1.80 mEq K per L of RBC per hour). By use of the radioisotopes a net gain of sodium was caused by a decrease in sodium output with the intake being little affected. The loss of potassium was caused by both an increase in output and a decrease in intake.

SCIENCE ON THE MARCH—LOBUND COMES OF AGE

Betty Appleton. Sci. Monthly, 80, 57-8 (1955).

A short review of the history of LOBUND (Laboratories of Bacteriology, University of Notre Dame) established by James A. Reyniers in 1928. At present there are three buildings: the Germ-Free Life Building, a machine shop, and an animal colony house.

The most widely known activity of Lobund is the raising of germ-free animals which are subjected to one and then another microorganism and the effects noted. Besides the Germ-Free Life Division there is the Biological Engineering Division which keeps germs in, instead of outside, the cages when specific germs are used. The third addition is the Microsurgical Division. Here microsurgery is performed on single cells with specially developed tools.

At present only germ-free insects (flies and cockroaches) are being studied. Investigation is being made on the relationship between microorganisms and the individual cells of plants and animals and the part that cells play in heredity.

THE EFFECT OF GLASS UPON THE ACTIVITY OF THE VARIOUS PLASMA CLOTTING FACTORS

Samuel I. Rapaport, Knut Aas, and Paul A. Owren. (Oslo University Hospital, Oslo, Norway.) J. of Clin. Invest., 34, 9-19 (1955).

The effect of quartz powder upon plasma clotting factors was measured in one-stage assays for antihemophilic A factor, antihemophilic B factor, prothrombin, proconvertin and proaccelerin. Proconvertin activity was increased three-fold or more; it was masked when Dicumarol was used and not activated in the presence of heparin although heparin could not "deactivate" previously formed active proconvertin. Antihemophilic B factor was also increased. No change in antihemophilic A factor, prothrombin or proaccelerin.

The possible mechanism for the glass activation of the antihemophilic B factor and proconvertin is the adsorption of a positively charged inhibitor onto the negatively charged glass.

CORTISONE AND CORTICOTROPIN IN RHEUMATIC FEVER AND JUVENILE RHEUMATOID ARTHRITIS

Joseph J. Bunim, Ann G. Kuttner, Janet S. Baldwin, and Currier McEwen. (Bellevue Hospital, New York.) J. Am. Med. Assoc., 150, 1273-8 (1952).

Anemia, an elevated erythrocyte sedimentation rate, increased concentration of serum fibrinogen and globulin levels, and the appearance of C-reactive protein (CRP) in the blood are among the nonspecific manifestations of rheumatic fever. A more sensitive index of rheumatic activity than the sedimentation rate is a precipitin test for detecting the protein that appears in the patient's serum during rheumatic activity and disappears when the inflammatory process subsides. All cases with acute rheumatic carditis treated with cortisone or corticotropin were tested at frequent intervals for C-reactive protein before, during, and after hormone therapy. Tests were strongly positive before treatment, only one did not become negative after a week or two after commencement of therapy, usually before the sedimentation rate reached normal. C-reactive protein usually reappeared when hormone administration was discontinued and when rheumatic activity continued the reaction persisted. During such therapy the disappearance of C-reactive protein is not indicative that rheumatic activity has subsided.

In some cases, clinical manifestations of the disease may not recur, but the sedimentation rate may rise or C-reactive protein may return but may not be accompanied by a rise in the antistreptolysin-O titer.

DETERMINATION OF C-REACTIVE PROTEIN IN THE BLOOD AS A MEASURE OF THE ACTIVITY OF THE DISEASE PROCESS IN ACUTE RHEUMATIC FEVER

Harold C. Anderson and Maclyn McCarty. (Hospital of Rockefeller Institute for Medical Research, New York.) Am. J. Med., 8, 445-55 (1950).

A precipitin test combining rabbit antiserum and the patients' serum in capillary tubes with subsequent incubation at 37° C. for two hours and overnight refrigeration at 4° C. is described. Antistreptolysin O (ASO) and titostreptokinase (A8K) titers were used also in studying the cases.

Results of the survey on the occurrence of C-reactive protein in patients with acute rheumatic fever gives perhaps the most sensitive indication of the disease process. In the early recovery period C-reactive protein may be absent when the sedimentation rate is much higher than normal. Other diseases with closely related clinical pictures such as rheumatoid arthritis and subacute bacterial endocarditis also are characterized by the presence of C-reactive protein. Once the diagnosis of rheumatic fever is made the test is of real value in the follow-up treatment as a measure of the activity of the disease process. It is probably the most sensitive test for the presence of rheumatic activity presently available.

AMONG THE NEW BOOKS

BASIC CARE OF EXPERIMENTAL ANIMALS: Prefaced by the Animal Welfare Institute. 350 Fifth Avenue, New York, N. Y., 36 pages. 11 illustrations. Free

Any laboratory using animals should have a copy of this brochure. The fundamentals of good care are outlined briefly. Not only are the physical aspects noted, but stress is placed upon the importance of their mental welfare. Disease Prevention, Parasites and Skin Troubles, Cleaning and Sterilization of Equipment, and Humane Ways of Killing Animals are sectional headings. There is a brief sketch on housing, bedding, feeding, handling, and general care of each of the most commonly used animals. A list of useful books and other references is included.

HEMATOLOGICAL TECHNIQUE: E. M. Darmady, M.A., M.D., (Camb.) F.R.C.P., Senior Pathologist, Portsmouth and Isle of Wight Area Pathological Service. Pathological Specialist R.A.F.V.R., Demonstrator of Pathology, St. Bartholomew's Hospital, London, and S.G.T. Davenport, F.I.M.L.T., Chief Technician, Portsmouth and Isle of Wight Pathological Service. Grune & Stratton. New York, N.Y., 1954. 4 color plates. 23 text figures. 197 pages. $3.00.

With its emphasis on the technical side of hematology, and compiled primarily for the student technologist, this volume may well be added to the library of the teaching laboratory. Brief explanations of the fundamental mechanisms behind the disease process are made. An outstanding chapter

is that on Errors in Hematological Procedures. Alternative techniques are given in many instances.

LABORATORY MANUAL OF BIOCHEMISTRY, 4th Ed.: by Benjamin Harrow, Ernest Borek, Abraham Mazur, Bilbert C.H. Stone and Harry Wagreich, Chemistry Department, City College of New York. W. B. Saunders Co., 1955. Philadelphia, Penna. 164 pages. 24 illustrations. $3.00.

As the title implies, this manual finds its primary function in the student laboratory. Several techniques are included for the first time, among these are the time course of starch hydrolysis, the estimation of catalase, etc. Details of a laboratory schedule for a two-semester course in biochemistry are given.

DIAGNOSTIC LABORATORY HEMATOLOGY: by George E. Cartwright, M.D., Assoc. Professor of Medicine, College of Medicine, University of Utah, Salt Lake City, Utah. 1954. Grune & Stratton, Inc. New York, N. Y. 104 pages. 28 tables. 5 figure illustrations. $3.00.

This very concise manual has the essential details of the procedures most commonly performed by a student or in the physician's office. The author has selected a single method for each determination, basing this upon "simplicity, accuracy, reliability, time of performance, cost, and availability of equipment." This volume is planned to be used with more complete references for supplemental procedures.

SECOND RADIO ISOTOPE CONFERENCE, 1954. PROCEEDINGS. VOL. I. MEDICAL AND PHYSIOLOGICAL APPLICATIONS. Edited by J. E. Johnston. Atomic Energy Research Establishment, Harwell, Berks, England, 1954. Academic Press, Inc., N. Y. 418 pages. 46 papers with discussion. Author index. $10.80.

The sections on Diagnosis and Biochemistry have papers of special interest to the technologist whose work is broadening into the radioisotopic field.

ANALYTICAL CYTOLOGY. Edited by Robert C. Mellors, M.D., Ph.D. Division of Experimental Pathology, Sloan-Kettering Institute for Cancer Research and Pathological Laboratories, Memorial Center for Cancer and Allied Diseases, and Graduate School of Medical Sciences, Cornell University, Medical College, N. Y. 1955. McGraw-Hill Book Co., N. Y. 9 articles. 511 pages, including oppropriate tables, diagrams and illustrative material relative to each paper. $15.00.

The purpose as stated in the preface "is to bring together in one volume some of the important contributions of physics and chemistry to the study and analysis of cellular structure and function." Emphasizing the role of the microscope in medicine this book gives special attention to newer developments in terms of both theory and application to biological problems. There are also articles on cytophotometric analysis in the visible spectrum, and x-ray diffraction techniques, with their application. Some of the more important histo- and cytochemical staining methods are reviewed. The eleven authors are all outstanding in their fields and bring together a most comprehensive collection of information.

BIOCHEMICAL INVESTIGATIONS: by John D. N. Naborro, M.D., M.R.C.P. Little Brown & Co., Boston, Mass. 299 pages. 6 tables of normal values, others in context. 3 figures. $6.00.

As background reference this volume is written primarily for the clinician. It shows how laboratory procedures can assist in the solution of diagnostic problems. Details of laboratory methods are not included. Those technologists who are continually expected to furnish more than self-evident reporting of test results will find it especially valuable.

AN AUTUMN GLEANING, Occasional Lectures and Addresses. By Sir Henry Hallett Dale, O.M., G.B.E., F.R.S., M.D., F.R.C.P. 1954. Interscience Publishers, N. Y. 225 pages. $4.25.

This is a collection of lectures from 1935 to 1952 to audiences not necessarily having special knowledge of the field of research in which the author

was engaged. Rather the appeal may just as well be to anyone who has a general enlightened interest in the progress of science. Some with a particular appeal to the technologist are "Viruses and Hererogenesis—an old problem in a new form," "the Freedom of Science," "Medical Research as an Aim in Life," "The Mechanism of Anaphylaxis," and "Science in Education."

Also received:

MANAGEMENT OF ADDICTIONS: Edited by Edward Podolsky, M.D., Department of Psychiatry, Kings County Hospital, Brooklyn, N. Y. Series of articles by authorities on Alcohol and Drug Addiction. 1955. Philosophical Library, N. Y. 413 pages. $7.50.

and

SEGAMENTAL ANATOMY OF THE LUNGS. By Edward A. Boyden, Ph.D. (Med. Sc.) Professor Emeritus of Anatomy, The Medical School, University of Minnesota. 1955 McGraw-Hill Book Co., N. Y. 276 pages, 125 figures, 12 color plates. 8 page Bibliography. $15.00.

Dragontek: The solution

Because of the laboratory findings, the presence of fetal hgb. (alkali resistant hgb.), the x-ray findings and the genetic aspects, the diagnosis of Mediterranean Anemia was made. If you are interested, this story was developed from a very excellent article on Mediterranean Anemia in "Blood," January, 1954. We recommend it. If you have never heard of alkali resistant hemoglobin, and who has, there is a reference theme.

From the *Newsletter*, November, 1954, Connecticut Society of Medical Technologists.

WHY I CHOSE MEDICAL TECHNOLOGY

By LIESELOTTE GARMS, M.T., *Little Rock, Arkansas*

It seems that many people wonder why I find my work so interesting. They do not see how fascinating and how much variety there is in the work of a medical technologist.

Being a daughter of a physician in Germany, I became interested in medicine quite young, and spent many after school hours with my father's technologist in the hospital laboratory. When I finished high school, I was convinced that it was a wonderful thing to help the physician in his work, and continued going to school for training in both laboratory and x-ray.

Never have I regretted my decision. I had to work hard, especially during the war, but there was always much satisfaction to be found in helping the doctors—and I ever strove for more precision, more accuracy in my work, which ultimately brought much responsibility. Newer developments and methods, different trends kept me busy pursuing more knowledge of my work.

With my husband and my two children, I came to the United States a few years ago—and I found out, with my profession, it was not too difficult to find work. After a few weeks adjusting to the new country and in a hospital laboratory, I was able to take a position in a doctor's office, and make my living—even with a very limited knowledge of the English language. The medical terms and chemistry are international, except for the pronunciation. A few names of chemicals are different, but the formula is always the same. Performing a test does not need any talking. "M-m-m" is international and takes care of a lot of conversation with patients without getting you in trouble. You soon learn your new country's language. But I didn't have to wait until I learned it to get a position.

Yes, I am glad of the choice I made in training to be a technologist. I would do the same if I had to do it all over. It is good to have such security!

The Arkansas Society of Medical Technologists.

From *the Techni-caller*, Vol. 3, No. 3, Fall, 1954.

LABORATORY TRAINING SERVICES

Communicable Disease Center
U. S. Public Health Service
P. O. Box 185, Chamblee, Georgia

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES

July 1955 — June 1956

Dates	Course No.	Courses	Duration
Sept. 12-23	8.40	Laboratory Diagnosis of Bacterial Diseases Part 1. General Bacteriology	2 wks.
Sept. 12 to Oct. 7	8.00	Laboratory Diagnosis of Parasitic Diseases Part 1. Intestinal Parasites	4 wks.
Sept. 26 to Oct. 7	8.41	Laboratory Diagnosis of Bacterial Diseases Part 2. General Bacteriology	2 wks.
Oct. 10-28	8.01	Laboratory Diagnosis of Parasitic Diseases Part 2. Blood Parasites	3 wks.
Oct. 17-28	8.20	Laboratory Diagnosis of Viral and Rickettsial Diseases	2 wks.
Oct. 17-28	8.50	Laboratory Diagnosis of Bacterial Diseases Enteric Bacteriology	2 wks.
Oct. 31 to Nov. 4	8.26	Laboratory Diagnosis of Rabies	1 wk.
Oct. 31 to Nov. 11	8.15	Laboratory Methods in Medical Mycology Part 1. Cutaneous Pathogenic Fungi	2 wks.
Nov. 14-25	8.16	Laboratory Methods in Medical Mycology Part 2. Subcutaneous and Systemic Fungi*	2 wks.
Nov. 14-25	8.55	Laboratory Diagnosis of Tuberculosis	2 wks.
Nov. 28 to Dec. 9	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	2 wks.
Dec. 12-16	9.40	Laboratory Diagnostic Methods in Veterinary Mycology	1 wk.
Mar. 12-23	8.20	Laboratory Diagnosis of Viral and Rickettsial Diseases	2 wks.
Mar. 26-30	8.26	Laboratory Diagnosis of Rabies	1 wk.
	8.05	Laboratory Diagnosis of Malaria**	2 wks.
	8.21	Virus Isolation and Identification Techniques**	2-4 wks.
	8.42	Typing of *Corynebacterium diphtheriae***	1 wk.
	8.51	Special Problems in Enteric Bacteriology**	2 wks.
	8.52	Phage Typing of *Salmonella typhosa***	1 wk.

* Completion of Course 8.15 or equivalent education or experience is a prerequisite.
** Courses offered by special arrangement only.

SCHOLARSHIP FUND

A fund, to be known as The Sister Alcuin Memorial Scholarship Fund, has been established in her memory at the College of St. Scholastica, Duluth, Minnesota. It will be administered through a committee of faculty members of the College and will be used solely to help deserving students become medical technologists. Donations should be sent to Sister Marybelle Leick, MT (ASCP), Department of Medical Technology, College of St. Scholastica, Duluth, Minnesota.

MOTION PICTURE FILMS

A completely revised Fourth Edition of "Professional Films" is now in compilation. It will include new sections providing biographical data on authors, and information on the audio-visual activities of medical schools, dental schools and graduate teaching centers.

AIM provides this valuable audio-visual information to the profession-at-large, without profit, as one of its contributions toward elevating the standards of medical and dental services by expediting the dissimination of professional knowledge. The listing will be of special interest to those who plan programs.

Write to the Academy-International of Medicine, 601 Louisiana Street, Lawrence, Kansas.